

Hunting the quangos

Britain's quangos should open themselves up more; putting them on the defensive will hardly help.

THE councils, committees, boards, institutes and so on, listed in the box, are all quangos. There exists no unique definition of a quango (originally a quasi-non-governmental organisation), but it may be taken that it performs governmental or public functions, or spends public money. It is not part of a government department, but it does have a dependent relationship with a department or with a minister for authority and/or for funds.

A quango-hunt is on at the moment; it is said that government ministers have been encouraged to see how many can be disbanded or trimmed. So it is valuable to have a report, *What's wrong with quangos?*, published by the Outer Circle Policy Unit (£2.00), which gives some background to the incredible diversity of quango that now exists. There are certainly serious questions to be asked of quangos — the report identifies issues of financial and political accountability, adequate provision of information concerning their activities and the secretive nature of much of the ministerial patronage that surrounds top appointments. In other words, quangos should be more open to scrutiny and accessible to the general public than they often are.

The question of who gets the plum jobs is a case in point. There is at present no provision whatsoever for most of the chairmanships of quangos to be advertised, nor (as the report points out) is there anything like adequate provision for an incompetent chairman to be brought to book. Furthermore many quangos have prestigious and influential councils where, again, the selection process is veiled from public view. And the council member's duties are most unlikely to contain any explicit instructions to

consult or inform a particular constituency.

Many of the ministerial appointments to quangos draw from the very British concept of a pool of 'the good and the great' — a list of worthy people said to reside in Civil Service Department filing cabinets (the list, that is, not the people). But this list is not comprised of those who at

Advisory Board for the Research Councils; Aeronautical Research Council; Agricultural Research Council; Animal Diseases Research Association; British Museum (Natural History); Centre for Environmental Studies; Computer Board for Universities and Research Councils; Electronics Research Council; Geological Museum; Health & Safety Executive; Inter-University Council for Higher Education Overseas; Medical Research Council; Meteorological Research Committee; National Biological Standards Board; National Computing Centre Ltd; National Institute of Agricultural Botany; National Radiological Protection Board; National Research Development Corporation; Natural Environment Research Council; Nature Conservancy Council; Rowett Research Institute; Royal Commission on Environmental Pollution; Science Museum; Science Research Council; Scottish Horticultural Research Institute; Scottish Society for Research in Plant Breeding; Social Science Research Council; United Kingdom Atomic Energy Authority; University Grants Committee.

various times have responded to advertisements or who have expressed a wish to involve themselves in policy matters — indeed, anyone who did so would probably be regarded as too pushy for appointment to anything. Rather, it is the product of a grapevine, and so unlikely to contain an adequate representation of those with radical views, those whose profession keeps them

remote from well-trodden paths, or those not yet forty.

Many (but not all) of the scientific quangos listed fulfil very necessary functions which would still have to be done (by civil servants?) if there were wholesale trimming, 'rationalisation' or cuts. To this extent they are probably fireproof. But quangos also have the merit of exposing outsiders to insiders and *vice versa*, and so should fulfil a vital role, in highly compartmentalised Britain, of providing at least a modicum of cross-fertilisation across the yawning chasms which exist between communities — academics, bureaucrats, industrialists, politicians. For this reason, there should be the greatest resistance to any move to replace quangos, particularly by civil service operations. However, there is still scope for quangos to improve their relationship with the outside world, both in terms of recruitment and in terms of the provision of adequate information on their mode of working. A wholesale quango-hunt will cause just the opposite to happen.



Universities accused of inefficient book-keeping

David Dickson reports on continued skirmishing between US politicians, administrators and scientists over use of research funds

Government auditors have accused Harvard University of claiming over \$2 million in "inappropriate" costs charged to federal research projects carried out by the university's School of Public Health between 1975 and 1977. They further state that, on the basis of sample studies of cost transfers, "we cannot attest to the propriety of about \$15 million charged to federal grants" during this period, out of a total of \$37.1 million audited.

These conclusions have emerged from an audit carried out by the Department of Health, Education and Welfare following allegations that the university was violating agreed procedures for claiming research costs from the federal government. The university has already announced its intention to challenge the draft audit, which has not yet been publicly released; but the incident has fuelled further skirmishing between politicians, administrators and scientists over how universities should account for their use of public research funds.

The politicians insist that, since the funds come from the public purse and their expenditure has been decided by Congress, then it should be demonstrated that they are spent in the way that Congress intended. But the universities claim that too much time spent accounting for the use of funds can hamper the research scientist and may ultimately undercut the value of the research itself.

Allegations about Harvard's misuse of funds were first made by Dr Phin Cohen, previously a research worker in the School of Public Health, in 1975. He repeated the allegations — and provided details of DHEW's draft audit — at a hearing held recently by the intergovernmental relations and human resources subcommittee of the House committee on government operations.

A particular focus of the hearing was a report just completed by the General Accounting Office claiming that many audits of university research expenditures were "not as effective as they could be". In some cases, inadequate university accounting systems meant federal auditors were unable to give an opinion on the allowability of costs charged to federal grants and contracts.

The GAO points out, for example, that of \$1.2 billion in research grants and contracts audited by DHEW in 1977, over a third was not properly documented. In one university, \$53.7 million of personal service costs, out of \$111 million charged to federal grants and contracts over a three year period, could not be adequately supported, since they were based on budget estimates rather than actual certified costs.

The GAO was also critical of some of the

DHEW's audit operations. Department officials, however, while accepting some weaknesses in auditing procedures, placed considerable blame on the universities which, they said, frequently failed to keep accounts in a form which meets federal standards. Mr Edward Stepnick, assistant inspector general for auditing at DHEW said compliance with auditing requirements was non-existent or inadequate at about 70% of the major colleges and universities audited.

Universities, in turn, continue to charge that the accounting requirements placed on them are often unreasonable and unrealistic. Their accounting is in fact, much better than they are being given credit for, according to Dr Max Binkley, vice-president for finance at Colorado State University. Publicity alleging scandal and malfeasance gave the public the wrong impression of issues which principally involved questions of accounting precision and documentation, he said. "A scientist is deeply engrossed in the pursuit of new knowledge in his or her field of specialisation, with the result that efforts towards precision in costing are disruptive and burdensome and constitute an impediment to the accomplishment of the ultimate objective."

All sides hope that the situation will be improved by the recent revision of Circular A-21, a document published by the Office of Management and Budget laying down guidelines for charging the direct and indirect costs of research. Considerable

differences still remain over costing principles, — particularly the way that research workers should account for the time spent on various projects — "the toughest and longest standing problem revealed by our audits" according to Mr Stepnick.

Under existing accounting rules, scientists receiving federal grant money have to report regularly on the way they have spent the previous month or two weeks. Under the revised A-21, a university can opt for a monitored workload system, in which the distribution of time is agreed before the project begins. Changes in the agreed workload have to be reported, and "significant" changes entered in the accounts. Mr Bowman Cutter, director of the budget in OMB, said this was a reasonable compromise, reached after considerable negotiation, between the demands of accountability and the flexibility needed by universities to administer research in an effective way.

University representatives, however, disagreed. They had been pressing that only *significant* changes in workload need be reported. "Insignificant changes to workload occur daily, perhaps even hourly," Dr Brinkley said. "Having to note all minor variations in activity was virtually impossible." He told the subcommittee that OMB's decision meant most institutions would not now adopt the monitored workload system, but opt instead for the personnel activity reporting, with which neither the government nor the universities were satisfied. "The overzealous attempt by the government to refine the system has evidently brought its defeat", he said.

Dr Thomas A. Bartlett, president of the Association of American Universities, told the subcommittee that his association wanted a number of changes in federal accounting rules. These included adoption of a monitored workload system "as a fully acceptable means for documenting effort", and grouping related grants for the purposes of administration. On the first, he echoed Dr Binkley's complaints that restrictions demanded by OMB in the revised Circular A-21 will probably deter widespread use of the system.

Dr Cohen suggested some more radical changes. He first described to the subcommittee how concern at the way university administrators had controlled the use of his grant money had led him to report the matter to the National Institutes of Health; following an investigation, the university had agreed to repay \$132,350. Dr Cohen suggested the "re-enfranchisement" of principal investigators, who would sign all time and effort reports, salary certifications and reports of expenditure, rather than delegating these functions to university officials. □



Harvard: sitting tight and challenging the audit

SRC tries apprenticeship scheme

DETAILED plans for two new experimental schemes aimed at improving the performance of graduates in industry are now being finalised by Britain's Science Research Council. The programmes, to be based on part-time studies, represent an unusual step by the normally academically-orientated SRC and indicate a strong feeling that British industry in general makes poor use of its scientific and engineering intake.

Although many of the UK's larger companies have adequate training schemes for developing the industrial potential of new graduates, many smaller firms make practically no arrangements for this. The two SRC projects aim to remedy the bias.

The first scheme, known as graduate initial education, will take the form of an apprenticeship package which will follow newly qualified students into industry. Through systems of tutorials, demonstrations and company-based projects over a two- or three-year period it will tailor their performance to the specific needs of their firms. A first experimental package, to cost about £100,000, is to be ready for student intake in October 1980.

The second programme will consist of

technological topping-up education for graduates of about 10-year standing who require up-dated instruction on new industrial practices. This will include the many managers whose educations cover only transistor electronics and not modern microelectronics. The scheme will aim at familiarity with a basic working knowledge.

In both systems, universities will probably undertake instruction, and may even award degrees if it is thought the schemes would otherwise affect staff-student ratios. However, in the case of the technological top-up programme, it is possible that the Open University, with its experience of popular presentation, may be introduced to instruct industrialists who might be alienated by a dry academic approach.

At the moment, both schemes are still at a rudimentary stage of planning and although they have been given the full go-ahead by the central council of the SRC, approval still has to be given by the Department of Education and Science as part-time higher education is not at present considered to be part of the council's remit. **Fine-tuning for technology, page 352.**

Robin McKie

Max Planck production venture fails

"We will concentrate future activities on our original function, which is to commercialise research results of the Max Planck Institute by licensing only," according to a spokesman of Garching Instrumente, a 100% commercial subsidiary of Max Planck Gesellschaft. The young enterprise has run into considerable difficulties, and finally has been forced to close down activities connected with the development and production of highly sophisticated instruments and pilot equipment — activities that accounted for nearly three-quarters of Garching Instrumente's turnover and employment.

MPG established Garching Instrumente in 1970 as a limited liability company whose aim was to commercialise technically useful research results of the Max Planck Institute in the industrial field. Stimulated by successful American ventures of a similar kind (from which the un-German name "Instrumente" was borrowed) Garching Instrumente was not only to deal with licensing (especially of Max Planck Institute patents), but also with the development, production and distribution of high technology hardware.

From its beginning, the new company raised considerable hopes and expectations, for it was considered to be the prototype of the young, small high technology venture needed in West Germany. The importance attached to it can be deduced from the remarkable efforts of the Federal Ministry of Research and Technology in the early 1970s to establish

a risk financing corporation specifically to assist risky high technology ventures.

In the first years of operation, at least, Garching Instrumente seemed to fulfil the high expectations. Turnover increased to DM4 million with around 24 staff; among its clients were big companies like Siemens AG, as well as small enterprises, research institutes and universities. About 75% of the licence agreements were contracted with the instrument, electronics and pharmaceutical industries.

As time passed, however, it became increasingly obvious that research work on the one hand and manufacturing business on the other were very different, and that switching from one to the other is not easy. The activities of the manufacturing division of Garching Instrumente seemed to be less and less in line with the expectations of the Max Planck Gesellschaft, and there were rumours not only of management deficiencies but also of losses higher than could be tolerated even in a newly founded venture. Now, as a consequence, MPG has closed down all development and manufacturing activities, dismissed the management and reduced the staff to a mere handful. Activities are now restricted to licensing.

The new management looks optimistically to the future: "The actual turnover out of licence agreements already exceeds DM1 million. That brings us near the break-even point of our present activities."

Klaus Höpfner

Maurice Bazin reports from Brazil on the return of the exiles for the country's major scientific conference

Scientists protest at US tycoon's Amazonian project

OVER 5000 scientists and social scientists congregated in the Brazilian north-east town of Fortaleza in mid-July for the 31st meeting of the SBPC, the Brazilian Society for the Progress of Science. The meeting's central theme was 'Dilemmas of Scientific Production in Brazil', reflecting the participants' preoccupation with the ways in which General President Figueiredo's Brazil is helping — or hindering — science. The problem of external dependency also lurked in the background as an ever-present spectre.

"The developed countries control science and technology, but they charge for letting us use it. And they charge dearly . . . It is enough to see the chronic deficit of the Brazilian balance of payments. Part of this deficit comes from paying royalties and patent rights; these are, in the last analysis, the price we pay for scientific and technological dependence" read an official note distributed by the general secretariat of the SBPC on the opening day. This point came up at a round-table on 'National Scientific Policy' which involved three representatives of agencies financing scientific research in Brazil. Physics Professor José Leite Lopes, visiting his country after ten years of exile, declared: "It is useless to discuss a science policy without discussing the forces which oppose its formulation. Shall we discuss scientific policy without discussing economic policies?" He then added that nothing could be done as long as the economic forces representing the interests of multinational companies determine technological and scientific production in Brazil.

The president of the SBPC reminded everyone that the Brazilian scientific community was never consulted in the past about national projects, and it became evident during the meeting that academics were ready to take advantage of the recently granted freedom of expression to criticise strongly the government's options. The relaxed atmosphere of the meeting (where mornings were left free to allow participants to enjoy the town's beaches) was interrupted, however, when local police arrested five student participants for distributing "subversive material"; a federal security agent declared later that "in addition, they all had taken drugs".

The hottest discussions centred on two huge ongoing projects: the Jari agro-industrial project in the Amazonian jungle and the nuclear agreement with West

Germany to build eight 1000 Megawatt nuclear plants in Brazil. Daniel Ludwig, an American citizen, owns a land area the size of Switzerland at the confluence of the river Jari and the Amazon. Investments in homogeneous forest plantation, a cellulose factory, cattle raising and rice growing on this site amount to \$700 million, and will reach \$1.5 billion in a few years when a paper factory is completed.

Banners reading "Amazonia is ours" were spread around an overflowing amphitheatre as Charles Briscoe, the American technician in charge of the Jari Project, explained that "Project Jari covers more than one million hectares in the State of Pará and the Federal Territory of Amapá . . . There are no boats loaded with gold or other riches leaving the country." But critics pointed out that all of Jari's activities are geared exclusively for export, and that the appropriateness of their technology is never considered. Agricultural production, for example, is fully industrialized: rice is sown by plane and tree planting and cutting are fully mechanized. Parts of Briscoe's presentation met with boos from the participants, several of whom denounced foreign control and the elimination of the indigenous Indian population.

West German nuclear deal heavily criticised

The government's nuclear programme was strongly criticised by Brazilian physicists on economic and political grounds; the technical and environmental arguments which dominate the nuclear debate in industrialised countries were not brought up. Professor Luis Pinguelli Rosa of the Federal University of Rio de Janeiro proposed a drastic revision of the contract with West Germany. He claimed that Brazil is in a position of strength: "Germany presently does not have other clients for its reactors. The only reason we did not succeed in imposing our conditions is because we owe \$40 billion to the industrialised world, and that forces us to follow a policy which is perfect for our creditors but not for ourselves." He also indicated that an official report by the Ministry of Planning recognises that the cost of electrical energy produced by nuclear reactors is three times that from hydroelectric schemes.

A longer-term view was presented by Professor Mario Schemberg, who trained some of the best known Brazilian physicists before he was forcibly retired from the University of Sao Paulo by the military government in 1969. He made an appeal for "freeing ourselves from the useless weight of the nuclear agreement with Germany, and cancelling the pledges which the military dictatorship made without consulting the people." He proposed an energy programme for Brazil for the next fifty years which would emphasise research in solar energy and nuclear fusion. □



Where have all the flowers gone?

THE savage destruction of the Brazilian rain forests has been dictated, to a large extent, by economic pressures over which the country's government can exercise little control. Yet this rapidly dwindling heritage of exotic plants and animals kindled the imagination of early explorers and continues to contribute to our understanding of our natural environment. In Brazil today the cultural and economic influence of the West is very apparent, and so it is strange to find little native interest in natural history reflected on the shelves of the big bookshops of cities such as Rio de Janeiro, Brasilia and Belo Horizonte. Despite a good deal of enquiry the only books I could find dealing with Brazilian wildlife at all were translations of glossy foreign 'coffee table' volumes.

Long searches through the second-hand markets uncovered only four more: a series published in São Paulo in the 1950s dealing with insects, birds and mammals; and a magnificent translation of an illustrated French book on Amazonian primates, published at the turn of the century — whose value was, unfortunately, well appreciated by the dealer. The Brazilian flora suffer a similar neglect, even though, at certain times of the year, one would be well advised to take some sort of field guide along when visiting the local fruit and vegetable markets.

Being able to name and classify species of plant and animal is an essential cornerstone of biological science and education. Once these basic elements can be identified, and their interactions understood, one may begin to appreciate the fragility of eco-systems such as the tropical rain forest.

It is disturbing, therefore, to find that wouldbe medical students, when asked to give the names of all the animal species they knew, could list, on average, only half a dozen — invariably creatures of medical importance such as the mosquito, the cockroach and the 'barbeiro', the bug vector of Chagas disease. Although this survey carried out by Professor Angelo Machado of the Federal University of

Minas Gerais, did not include questions on plant life, my own experiences suggested that here the situation is even worse. Indeed, one graduate biologist I spoke to was surprised to discover that natural selection occurs outside the animal kingdom!

Machado is a well respected morphologist and neuroanatomist, whose spare time pursuits include some most elegant studies on the ecology and taxonomy of neo-tropical dragonflies. His wide interests include conservation — he is a founder member of one of the few environmental pressure groups in the country — and, more recently, an attempt to understand the lack of interest in wildlife which is apparent in the country's development of its natural resources. Brazil's politicians and administrators, he feels, are 'frightened by nature', and so he has turned to studying the development of the concept of the 'forest' in school children throughout the country.

This survey is still in its early stages, but Machado has already found that the child's concept of the forest, and its association with fear, begins to form early in life. It seems to be based on nursery rhymes and children's stories imported from Europe and North America, which emphasise the dangers of going into the woods.

In the younger child this ignorance can be easily counteracted. Drawings and paintings of the 'forest' made by 7-10 year olds commonly included aeroplanes spraying the undergrowth with bullets and napalm; but after the same children had been taken by Machado on a trip to the real forest these were replaced by birds and butterflies, and the children paid far more artistic attention to leaf forms and flowers.

At the present rate of development Brazil's forests may only survive for another 30 years, but if Machado's children can be so easily convinced of its value there is some hope that this new generation may allow it to stand for longer. The final decision may perhaps lie with the teachers rather than the politicians.

David Bousfield

news in brief

Third World space science institute proposed: An International Institute for Space Science and Electronics, where scientists from developing countries can carry out front line research in radio science, electronics and space applications, may soon be set up in Kenya. Establishment of the institute was strongly recommended by a recent workshop in Ooty, India attended by scientists from ten developing nations and organised by the Tata Institute of Fundamental Research's Radio Astronomy Centre. The new institute's first project will be the construction of a \$20 million Giant Equatorial Radio Telescope (GERT). Nobel Laureate Anthony Hewish of the UK Cavendish Laboratories, consultant to the workshop, said he was strongly in favour of the project which he thought was "good and sensible". GERT will be the world's earliest warning system for geomagnetic storms and will also carry out pulsar research, make rapid surveys and give information on the structure of radio galaxies. According to Hewish, GERT's research is unlikely to be repeated in other countries because it will use wavelengths of around 300 MHz whereas most other research in this area tends to use shorter wavelengths. Developing nations should be able to finance the project themselves using technology within their capabilities. India, Kenya, and Nigeria have expressed strong initial interest in the project.

US trade unionists oppose expansion of nuclear power: Twenty US trade unionists, including the presidents of two international unions, have issued a statement calling for an end to the development of nuclear power. They accuse the nuclear industry of using "scare tactics" in threatening that such a move would cause widespread unemployment. Attitudes towards nuclear power within the US trade union movement are sharply divided, largely because the construction and operation of nuclear power stations provides many jobs for the members of a number of powerful unions. However the recent letter, represents a growing awareness among trade union members of the hazards as well as the advantages of nuclear power.

Of the 20 signatories of the statement, five are union members who live and work near the Three Mile Island nuclear plant. In addition to ending the development of nuclear power, the statement demands: guaranteed new, safe jobs at full union wages, with paid retraining to all now employed in nuclear or nuclear-related industries; a substantial shift of investment resources and workpower to conservation, clean and safe coal, and solar energy; and local, democratic control of energy sources, production and distribution.

Computer scientists continue strike: Computer specialists in the telecommunications section of the UK Postal Service voted overwhelmingly last week to continue their selective strike in the telephone billing department. They also voted to censure the leadership of their union, the Society of Civil and Public Servants, for recommending acceptance of management pay offer of 18½%. The union has been on strike since March in support of a pay claim for parity with professional engineers in the Post Office and with administrative grades in the Civil Service Department. The strike has been joined by the Civil and Public Service Association representing 34,000 clerical grades including computer operators. A total of 100 people are on strike and are being supported by a strike levy on the 40,000 members of both unions.

Academy panel calls for new "microstructure science" research programmes: A panel of the US National Academy of Sciences has warned that the development of micro-electronic technology appears to be outstripping the basic scientific knowledge on which such technology depends. It recommends the setting up of a

number of new research programmes, primarily in universities, in fields that it calls "microstructure science and engineering". The academy panel also encouraged greater collaboration between university and industry laboratories, as well as an increase in programmes of non-proprietary research and development in industry. *Microstructure science, engineering and technology.* Solid State Sciences Committee of the National Research Council.

Getting steamed up over waste heat: Combined heat and power (CHP) could account for a third of domestic heating next century, according to a Department of Energy report published last week. The report estimates that CHP saves between 2% and 9% of all primary energy consumption when it is fully operative by utilising waste heat from modern power stations. Extracting this heat at high enough temperatures provides space heating and hot water for domestic, commercial and public buildings. CHP is likely to be a viable economic option for district heating in areas with a net heat load density above 15 megawatts per square kilometre and so can be used for 80% of the heat load in Greater London, West Midlands, Greater Manchester, Merseyside and Glasgow. CHP's potential role in industry is limited since 70% of electricity generated is already associated with heat recovery. For CHP to be successful by 2000, investment must begin immediately, and it will have to compete with other fuels, particularly gas, for funding during the next 10-20 years. *Combined heat and electric power generation in the UK.* HMSO £3.75

French agronomists protest over potential redundancies: Scientists at the Institut National de la Recherche Agronomique, an organisation which supports most of French agronomy research and employs 7,000 people, recently locked up their director-general for several hours in an angry protest against a change in the institute's status that could lead to redundancies. The French government intends to change INRA into a group of specialised subsidiaries to exploit commercially certain results of agronomy research. The government is concerned about the increasing penetration of the French agricultural and food processing industries by British and Dutch interests. The trade unions involved see two dangers in the proposal: a reduction in fundamental research in favour of more development oriented research and less security of employment. INRA's researchers currently have the status of *fonctionnaires* (civil servants) and under the modified statutes they would lose the guaranteed employment that they now have.

What changed Sir Richard's mind? The government appears to have found a respectable volunteer to fly a kite for its plans, already mentioned in the press, to eliminate some of the less prestigious universities and polytechnics. Sir Richard Way, the Crown-appointed principal of King's College, London, who previously ran London Transport, made an astonishing *volte-face* in his speech at the recent exhibition to celebrate 150 years of the college. A press release prepared before the opening ceremony contained the unsurprising claim that new cuts in university expenditure would "probably make it impossible for the British university system to maintain the high standards at which it had always aimed."

However, when Sir Richard arrived in the company of the Education Secretary, Mark Carlisle, to open the exhibition, he announced that he did not in fact consider the universities as a whole to be short of money (12 July, page 97). Sir Richard went on to hark back to his days at the War Office when a shortage of money led to a painful reduction in the number of battalions by a process of amalgamation. Sir Richard made it clear that he considered a similar solution appropriate to higher education so that centres of excellence should not suffer.

The Israel-Egypt peace treaty has opened up the possibility of scientific links. Here, 'Nature' correspondents describe the views from Cairo and Tel Aviv

Egypt: divided and uncertain

THE Egyptian scientific community is divided on the issue of collaboration with Israel. A small number of high ranking scientists see many advantages in undertaking joint projects; they argue that the Israelis have certain expertise in research areas that are important to Egypt, and Egypt should take advantage of the Israeli offer to collaborate in these areas.

There is, however, considerable opposition to this view from the rank-and-file scientists. Their argument is that the essential need of Egypt is not expertise but funding. If Israel can fund Egyptian research ventures, all well and good; but that's where collaboration ends. The Americans and Europeans already occupy positions of prestige in Egyptian scientific establishments as advisers and consultants, and many Egyptian scientists fear that Israelis will be added to the list. And they consider this not so much collaboration but more a form of domination.

Over the last few years, the Egyptian scientific community has been looking more and more towards the Arab OPEC countries as a major source of funding. A number of Egyptian projects in solar energy, desalination, oceanography, pollution studies and agriculture are already being financed by some of these countries, while Egyptian scientists and technicians form the backbone of industry and academia in Saudi Arabia, Kuwait, Bahrain and the United Arab Emirates. Despite the Arab boycott of Egypt, these scientists continue to work in Arab OPEC countries, many joint projects still continue, and these countries continue to recruit scientists and technicians from Egypt. The expectation of further collaboration with Arab OPEC countries is, therefore, much stronger than the possibility of co-operation with Israel. Many Egyptian scientists believe that co-operation with Israel will seriously damage the possibility of further collaboration with the Arab OPEC countries.

Dr Fahamy Ramadan, Secretary General of the National Research Centre of Cairo, sums up the feeling of many Egyptian scientists when he says: "All Arabs are brothers. Brothers fight; but they do not stay divided for long. They eventually make up. Our Arab brothers will make up with us, and much sooner than most people think". It seems that most Egyptian scientists would rather wait for their "Arab brothers" to make up with them then actively collaborate with Israel.

Ziauddin Sardar



Sinai 1967: can the battlefield now become a symbol of peace and co-operation?

Israel: a one-sided love affair

DISCUSSIONS about scientific and technological co-operation between Israel and Egypt have so far been very much like a one-sided love affair; while dozens of Israeli professors and politicians have spoken about the potential benefits of such co-operation little response has been forthcoming from their counterparts in Egypt. To be sure, there have been unofficial meetings between top Egyptian and Israeli scientists in third countries like the US and Germany usually on the initiative of well-meaning intermediaries. But so far the Egyptians have avoided public statements on the subject.

Yet the Israelis go on hoping and talking. The most dramatic presentation of a co-operation scheme was made some two months ago during Sadat's visit to Beersheba by Joseph Tekoah, former Israel ambassador to the UN and new president of Ben-Gurion University of the Negev. Speaking at an internationally televised campus ceremony, with Begin and Sadat on either side, Tekoah announced that the university had decided to establish a \$100m fund for desert research of potential benefit to both Israel and Egypt. Egyptian scientists, he added, were invited to participate in the research projects and in the fund's administration. Sadat was all smiles, but that — so far — has been the only Egyptian response.

Slightly more guarded was the recent statement by Haifa Technion president Amos Horev, who expressed the Technion's willingness to enter into bilateral relations with Egyptian institutions. As a first step he expected that Egyptian technological and science university graduates might study for higher degrees at the Technion, and that Egypt might wish to utilize the Technion's research laboratories and testing facilities. But under no circumstances, Horev warned, should Israelis delude themselves into thinking that they were going to 'save Egypt'.

The most logical place for co-operation to begin would be the Sinai peninsula, where for the last 12 years Israeli scientists have done extensive research, the results of

which are now being turned over to the returning Egyptians. This is by no means a new idea, as a number of American Presidents, beginning with Eisenhower, have suggested the establishment in Sinai of joint Israeli-Egyptian power stations and desalination plants. Before this scheme was finally shelved, scientists working at the Oak Ridge National Laboratory under Dr Alvin Weinberg had produced a detailed report on how it could be carried out. They envisaged the installation of two reactors with a combined output of 1000 MW, which would provide power for new industries producing chemicals, fertilizers, plastics and aluminium, as well as for desalinating enough water to irrigate 300,000 acres of land in Egypt and the Negev desert.

These plans still exist, as do new ones engendered by the euphoria of peace. Yet the Egyptians remain reticent, as is evident in the experiences of an Israeli scientist who has just returned from a visit to Egypt. He travelled there on a second, non-Israeli passport, but his hosts, top figures in the Egyptian scientific establishment, know that he lives in Israel, as does anyone who reads his papers in the journals. Behind closed doors the Egyptian scientists spoke enthusiastically about the possibility of co-operation with Israel in spheres like agriculture, medicine, solar energy and desalination. Yet when someone came into the room, such discussions stopped and the guest was introduced as being from Europe.

In order to overcome this apparent Egyptian hesitance about bi-national co-operation, leading Israeli scientists have suggested that international organizations should sponsor large scientific conferences in Israel and in Egypt, where it is hoped there would be massive participation of Israeli scientists when the meeting took place in Egypt, and of Egyptian scientists when it was in Israel. Moreover, people here place great stress on the need to choose topics where the Egyptians excel and can teach the Israelis, as well as fields where the opposite situation exists.

Nechemia Meyers

Hungary steers a course without UN aid

Hungary's Academy of Sciences is now working out its Five Year Plan for 1981 — 1985, the first in which it will be entirely without the support of the United Nations Development Programme. For although by 1975 the Hungarian economy had passed the formal criterion for a 'fairly well-developed country', careful management of the UNDP funding ensured a certain carry-over beyond the official termination date. Furthermore, careful negotiation with the UNDP administrators cut down the expenditure on administrative overheads, so that an unusually high proportion of the funds available actually went on the equipping of Hungarian science, rather than on the expenses and infrastructure associated with the presence of international 'experts'.

A leading figure in these negotiations was Dr Bruno Straub. A biochemist of some renown, since the mid 1960s he has had an impressive second career as an administrator for scientific projects. In 1969, he was appointed a Vice-Chairman of the International Atomic Energy Agency committee for working out a nuclear safeguard agreement, and after the withdrawal of the chairman, Kurt Waldheim, from active participation, he took over as *de facto* chairman. In 1971, he was elected to the executive board of the International Council of Scientific Unions, serving on its Environmental Commission. And in his native country, Dr Straub applied his skill and renown as an organiser of science to a problem which, as a biochemist, he felt to be a major priority — the sad state of Hungarian biology.

Since the Hungarian economy is considerably dependent on agriculture, the development of the country's biological expertise seemed to him a matter of pressing concern. Unfortunately, in the mid-1960s, Hungarian biology was firmly in the hands of what Dr Straub described as "the old people". These, he explained, were "biologists and zoologists, only interested in systematics and taxonomy". Such an attitude, Dr Straub explained, found a certain support among the administrators. Not for ideological reasons — Lysenkoism never took hold in Hungary and the principle of a free inflow of information always held good.

The problem was simply one of finance. "You can do taxonomy cheaply, but experimental biology means instrumentation. You can get simple things here in Hungary or else from Poland — but sophisticated work means dollars; and that means a special decision of the government."

By tapping the UNDP aid to Hungary (about \$1 million per year over five years), it became possible for the Academy of Sciences to construct and equip a modern biological research centre at Szeged with

four institutes — biochemistry, biophysics, plant physiology and genetics, with a fifth institute, enzymology, sited in Budapest. Between 1973 and 1978, the Szeged Centre received some \$1.2 million from UNDP, more than 50% of which was spent on equipment, with the participation of foreign experts reduced to "just those who could really help us".

In return for this special arrangement, Hungary had to undertake to give scholarships at Szeged to trainees from developing countries, to whose problems Hungary is still fairly close. Dr Straub, who had accepted the post of General Director of Szeged in 1973, on the understanding that it should be a rotating leadership between the five sections (he resigned in 1978), was able to persuade the Academy of Sciences to support this scholarship scheme. "While the UNDP money was still coming in, they were quite keen about it", he explained, "now they are a little less happy, but still, the scholarships continue". Luckily for intending applicants, the working language at Szeged is English, and the third-world trainees at present include representatives of Vietnam, Korea, a number of African countries, and (so goes the Szeged joke), a citizen of Lancashire.

Research at Szeged varies from basic investigations of immunological reactions in blue-green algae, through studies of the genetic basis of nitrogen fixation (with relation to lucerne, Hungary's most important legume), to contract research funded by agricultural concern. According to Dr Farkas, Director of the Institute of Plant Physiology, such contract work can raise the salary of those involved by up to 10 to 50% — though not, unfortunately, his own, since Directors and Vice Directors of Academy Institutes are not allowed to accept contract payments. The government, he explained, is trying to bring in material incentives (ie bonus payments) in a number of aspects of scientific life, both for economic reasons ("it works out cheaper in the long run") and also to stimulate creativity. He himself was not entirely happy about the contract scheme, he said, pointing out that many people would prefer to see a regular increase in basic salaries. Work for the agricultural cooperatives, such as the current project on the technology of vegetative propagation of vine-stocks, was "a burden, but beneficial". Nevertheless, he intimated that he would not be sorry if the Academy were to reverse its present policy on bonus payments, by setting a limit as to how far an individual scientist can augment his salary. Otherwise, there might be a real danger that scientists would be distracted from their main line of research.

Whatever the temptation at the laboratory-floor level, Hungarian science

does not intend to abandon fundamental research. According to Dr Istvan Lang, Deputy General Secretary of the Academy of Sciences, fundamental research accounts for some 14% of Hungary's total R & D costs. "We think we must keep it at this level, and the state budget gives it a long-term guarantee."

There is no question of a cut-back; the main problem is what sort of fundamental research should be carried out. Hungary, he said, with about 0.9% of the total world capacity for research, clearly must be selective, and has tended to concentrate on fields in which Hungarian scientists had already made a name — mathematics, physics, and biochemistry. Within these fields, there is a certain amount of "target orientation". For example, Hungary wants to develop the pharmaceutical industry. "Hence we hope that a young biologist will choose his work with this in mind, rather than getting attracted to space biology, say!" (Though Hungary does, in fact, have a space biology programme, as well as a five year project at Szeged on the asymmetry of living matter, now being phased out in the words of its director, "until we find another living system or make life artificially, to provide material for comparison".)

Responsibility for science planning is divided between the Academy of Sciences and the National Committee for Technical Development, both of which are subordinated to the Government Science Policy Committee, headed by Gyorgy Aczel, one of the Deputy Prime Ministers. Policy decisions taken by the Science Policy Committee are binding on the Academy, Secretary Lang explained, "But what you have to understand is how our planning system works. We are a centrally planned economy, but we don't plan everything centrally."

As an example of academic freedom, Hungarian style, he cited his recent work for a special council to decide on future development of the Lake Balaton area. "When I was elected head of the Council, I chose as our first task the evaluation of all existing data, and the preparation of recommendations to the Government. These were discussed and approved by the Council for the Environment and Nature Conservation. Next a regional development plan was elaborated on the basis of these recommendations".

"Our forecasts shocked public opinion," he continued "We had to point out that if nothing changed, if the input level remained the same and the rate of control the same, then in 15 years' time, Balaton would be green with eutrophication and unacceptable as for leisure use".

It is, perhaps, not a matter of great surprise that the new plan was accepted on June 10.

Vera Rich

Fine-tuning for British technology

— or will political pressures put a spanner in the works?

The Finniston report on engineering and education could be crucial for the UK's industrial revival, but its proposals may run counter to Conservative policy. **Robin McKie** reports

BRITAIN'S recently-elected Conservative government is facing a dilemma that could expose basic contradictions between its much-publicised antipathy towards public spending and its equally well-proclaimed desire to boost the country's industrial growth. The predicament will come in the form of the Finniston report, a major policy document now being completed by leading industrialists and educationalists, which will recommend a package of proposals for regenerating scientific and technological impetus within the UK's manufacturing industry.

At the centre of these plans lies the proposed formation of a British Engineering Authority which would take over engineering education, training and research throughout the country. Such a body would also be responsible for the registration of engineers, in the same way as the General Medical Council handles the registration of all UK doctors, and may even take on the role of granting licences for engineers wishing to act as industrial consultants.

However, given the new administration's recent moves to scrap a thousand similar 'quasi-autonomous non-governmental organisations' (or quangos as they are affectionately known), it is hard to envisage them allowing any increased public control of the private sector — particularly in view of the Conservatives' election pledge to leave industry alone "to get on with the job".

Corrective measures are badly needed, nevertheless, for it is now widely accepted that Britain's manufacturing industry is not as competent, efficient or productive as our foreign competitors. In particular, companies are accused of very poor intakes of scientists and engineers into management — a tendency which discourages further gifted and ambitious students from such a career route. This means a resulting intake of poorer engineering and technological students, with even worse prospects of reaching management — and so the spiralling problem becomes more critical.

Indeed, it is a crisis recognised by the new Minister of Science, Mr Neil Macfarlane, who believes that UK industry has shown scant regard for scientific and engineering talent in the past. "I think it is greatly to be regretted that the engineer and the scientist are generally relegated to a secondary level in the hierarchy of any industrial organisation in the UK. We have been

taken over by financiers and lawyers", he said in a recent interview with *Nature*.

With such difficulties in mind, the previous Labour government set up the committee of inquiry into the engineering profession, led by Sir Monty Finniston, head of Sears Engineering and former chairman of the British Steel Corporation. Its task was to review:

- British industry's requirements for supplies of engineers;
- The role of engineering institutions in controlling standards of qualifications;
- The advantages and disadvantages of registration and licensing of engineers;
- Comparable arrangements in other countries, particularly in Europe.

But Sir Monty and his committee deliberately chose to interpret this remit in the widest possible terms and have set about preparing an entire blueprint for ensuring industrial regeneration in Britain. This is not to be done on the macro-level of gross economic distortions (as in recent budgets which have attempted major inflations of markets and profits), but by tinkering at a micro-level to fine-tune the country's industrial engine and prepare it for improved performance.

A central part will be played by the proposed British Engineering Authority which will take over the role of registration of engineers. At present this task is performed by the Council of Engineering Institutions, a central body made up of representatives of the country's many engineering institutions (such as the Institution of Electrical Engineers and the Institution of Mechanical Engineers) which specify, approve and enforce the minimum standards of professional conduct. Many now consider the CEI to be a weak, ineffectual body which merely awards chartered engineering status to anyone with a relevant degree at ordinary pass level. Instead, the new authority, which would probably include lay representation, would operate as a strong independent agency that would restore public confidence in the profession.

The new system would control the awards of titles — 'qualified engineer', 'qualified engineering technician' and a specially-created elite called 'diploma engineer' — only to those with sufficient training, experience and education. This would allow tight control over engineering practice by ensuring that those who fell below standard would lose their titles, and would make it an offence for them to



continue to use these titles.

The diploma designation would represent a move towards creating an elite similar to the German 'superengineer', although this is more an attempt to boost the status of UK engineers than a bid to create a particularly practical type of engineer.

A further controversial plan being considered by the Finniston committee is a proposal that all university and polytechnic engineering courses should be closely scrutinised by the new authority — and that institutions failing to meet basic standards should be stripped of financial support. At present such an accreditation body operates in the United States, but it has no financial influence over university departments and only recommends to potential students and future employers those courses it believes are satisfactory. In considering this new, and far more radical proposal, the committee will be suggesting that the University Grants Committee and the Science Research Council be stripped of their present control of funding for engineering education and research — a move that would no doubt be strongly contested.

Indeed the proposal has already been

rejected by the main committee, but the sub-group on registration and licensing has since insisted that it be re-considered, stressing that a bold measure is necessary to swing education behind the interests of manufacturing industry. "We think that there are forces of inertia in higher education that will stand in the way of a reform of engineering education unless leverage is exerted", stated a recent report from the sub-group to the main committee.

A further consideration that may influence the Finniston group is the likely constraint on future education spending. As increased resources are especially vital for engineering, the committee may recommend that the education sector is no longer a suitable outlet for funding and suggest a more fundamental alternative. This argument could find particular favour with the Conservatives, as it proposes moving money away from traditional public spending areas and closer to the interests of private industry.

The proposed British Engineering Authority would also monitor training schemes for graduates entering industry. At present, it is well-known that many smaller firms offer inadequate and inferior training programmes and such students would be at an immediate disadvantage upon the introduction of a strict registration system. This has been one important motivation behind the UK Science Research Council's launching of an experimental programme — to be known as graduate initial education — which will next year offer part-time postgraduate university-based instruction in industrial subjects for science and engineering graduates.

Thus it is envisaged that the BEA will maintain a strong hold over the flow of engineers into industry. Basic education would be monitored through an accreditation system; training would be controlled by close scrutiny of companies; and the actual quality of manpower would be governed through a tight registration system.

At the top end of the market, quality would be supervised by operating a licensing system for consultant engineers who offer special expertise in particular industrial areas. This would ensure the quality of those engineering experts who advise companies and public bodies and would act as a further qualification on top of registration.

These moves all reflect recognition that British industry is flagging not just because of poor investment in modern technology but also because it fails properly to utilise available talent and intellect. And even when this problem is overcome, systems will still have to be devised for professional, practising engineers to keep abreast of current technological developments. For example, many UK managers have an education which covers only transistor electronics; their lack of knowledge of modern solid state physics

can lead to fears and prejudices about working with complex microelectronic technologies. The solution probably lies in various forms of continuing education courses, to be taken every five or ten years.

The extent of this problem has already motivated the Science Research Council to arrange technological topping-up courses — based at universities — for graduates of about ten years standing, and these are soon to be introduced into the UK. The Finniston committee is likely to favour a similar scheme which would also include grants for fares, fees and books.

Other proposals to boost the quality of personnel in industry will include methods for helping already-trained women engineers to get back into industry, and ways of improving attitudes towards industry in school classrooms. In this latter area, a particularly favoured plan is for interchanges of staff between schools and major manufacturing companies.

But such a scheme would not mean simply recruiting teachers from former industrial personnel — they may have left the commercial sector because they failed to operate adequately there. Instead a system of rotation of roles is seriously being considered, although it is acknowledged that this could cause difficulties with the trade unions involved.

In outlining these various proposals, the Finniston committee has recognised that one major problem has been the vast expansion of the British university sector in the early 1960s. This has led to colleges being elevated to university status, producing a more academic approach and forcing them away from local industries with which they previously had strong ties.

Now the Finniston committee is considering setting up regional engineering centres — to be run by the BEA — which would provide a mixing ground for academics and industrialists. The centres would be based at universities but would have the explicit roles of co-ordinating and generating engineering research, applications and training in relation to the particular industrial needs of a region. Such institutions could be set up at Imperial College (London), Aston (Birmingham), Leeds-Bradford, and Glasgow-Strathclyde universities.



Monty Finniston: due to report later this year

Another problem caused by university expansion has been the diverting of most engineers into degree courses meant for chartered engineers — those concerned with the innovation and creation of new technologies. The old college and part-time routes which provided the country's many technician engineers (those concerned with maintaining existing technology) are now virtually extinct.

As a result students who have received chartered engineering education have ended up totally unprepared as technician engineers. Finniston may consider introducing a new technician engineering degree course at universities to provide the most suitable education for those aspiring to the proposed 'qualified engineering technician' status.

At the moment, there is still strong agreement over the various issues between the 18 members of the committee and it is unlikely that a minority report will accompany the final publication of the full Finniston document. Indeed, one member, although slightly critical of the over-representation of academics and "fringe people" not directly concerned with industry, considered the committee had shown "a remarkable working chemistry" over the past 18 months.

The strength of this chemistry is likely to be tested in the new few weeks as work on the report is completed — for the committee is now under strong pressure to water down its recommendations, particularly from some engineering institutions who view a strong independent authority as a threat to their power and independence.

And within the committee, there are some signs of resolve weakening. In particular, there is now talk of finding a role within the BEA for the much-vilified CEI (because it has had past experience of controlling standards of education and training for engineers), although it had previously been agreed there would be practically no room for the CEI in the new authority.

This is only a small indication, but it could spell a warning that Finniston, in the hope of preventing undue reaction and prejudice to the report, may fudge the available options. In turn this could give the Government an excuse to further water down the proposals, eventually rendering them totally ineffectual.

Such a dilution of Finniston's proposals could have tragic consequences for Britain. The country's industrial slump can be blamed squarely on the failure to take up technological innovations and to make use of scientific and engineering talent. No amount of tinkering with sterling revaluations or credit markets will alter this, and strong remedial action is urgently required. The Finniston report — to be published some time between September and December — represents the Government's chance to change Britain's future technological development for the better. □

correspondence

Airey Neave Memorial Trust

SIR, — Many people have suggested to us that they would like to commemorate the life of Airey Neave, assassinated this year by a bomb in the Palace of Westminster (for obituary see page 433). Some have sent money now banked in a special account.

Since Airey Neave believed in the supreme importance of freedom under the law, we consider that we can achieve something that he would have held dear by creating a Trust to provide for research into the extent of personal freedom under national laws. This may include the provision of travelling scholarships. Candidates will be chosen after they have submitted a short synopsis of the scope of the paper they intend to write. The papers, embodying their research, will be published.

In promoting these studies, priority will be given to applications by candidates from fields in which Airey Neave had a particular interest; former pupils of Radley College or Abingdon School and graduates of Merton College, Oxford; holders of degrees in science or law; members of the legal profession; former prisoners of war; authors; Members or former Members of either House of Parliament.

The value and number of such scholarships will depend upon the amount of money received. Application has been made for the Trust to be registered as a Charity.

Those who wish to be associated with this appeal should send their contributions to The Airey Neave Memorial Trust, c/o The House of Commons, London SW1. Covenant forms will be available on application to the Trust.

Yours faithfully,

JOHN TILNEY (Chairman)
and the committee of the Trust.

London SW1, UK.

Costs of toxicity testing

SIR, — That ICI Ltd is spending £6 million per annum on its toxicity testing programme seems to underline the fears of smaller companies that the tests they are asked to undertake are too expensive. This is even more the case if we concede that these tests can be but a crude guide to the possible hazards to man. Still, the bankruptcy of a few firms and a reduction in innovation might be a small price to pay for the sensation of safety we all crave; in any case, as the new legislation applies to new chemicals, only the larger more affluent companies with new product developments are likely to be much affected.

Or are they? Mr Langley has suggested (5 July, page 9) that industry ought to be doing more, anyway, under the 1974 Act, and the new notification scheme merely records that defined work has been completed successfully. Is there an implication here that existing chemicals will be subject to the same testing arrangements? The logic of the Act suggests this ought to be so — and recent experience with monomers has thrown doubt on 'long manufacturing experience' as a safeguard on health, making it more likely that some experimental results will be needed.

In this case, the economic impact could be substantial and, because it is happening at a time when another world recession is expected, it could have even more profound effects than

usual. It is time to establish some priorities. Let the Health and Safety Executive produce guidelines, not on how to test chemicals, but on how to select chemicals which require to be tested — unless they intend to deal only with new chemicals. Let us concentrate our resources on developing test procedures which effectively screen chemicals for the properties we want to avoid at all costs; and leave lesser hazards until we can afford to attack them. Above all, let us recognise that life is a hazardous and invariably fatal process which will surely survive twentieth century industrialism, if we keep our scientific 'cool'.

Yours faithfully,

D. M. CONNING
British Industrial Biological Research
Association, Surrey, UK.

Attack on paranormal is unscientific

SIR, — It is surely profoundly unscientific to claim that "neither electro-magnetism nor any other scientific theory can explain" ESP phenomena as Taylor and Balanovski do (14 June, page 631). The most anyone can ever claim is that existing theories do not explain a phenomenon so far as they can see — someone else may be clever enough to find an explanation within existing theories, or to discover a new explanation. The latter possibility is not "ruled out" by the "successful reductionist approach of science," but is probably the main attraction of science to its practitioners. To go on to deduce that an argument of this sort casts any light on whether the phenomenon exists is even more bizarre — would they have argued against the heat of the Sun before relativity and the theory of fusion? Or do they now assume there is doubt that the mu-meson is heavier than the electron because no-one has yet suggested an explanation?

It might be instructive for these authors to study the history of the famous argument over the age of the Earth at the end of the last century. Kelvin had calculated the age from the time taken for cooling; the result was far too short to allow species to have evolved. Biologists suggested Kelvin might have ignored some unknown source of energy, and were roundly mocked by the physicists for being "unscientific". The fact that Kelvin's proponents were both confident and arrogant did not prevent them being wrong — as the subsequent discovery of radio-activity proved.

I might add that I do not believe there is much evidence for ESP, but this is based on the absence of unambiguous experiments rather than on anyone's inability to think of an explanation.

Yours faithfully,

M. WHIPPMAN
London SW20, UK.

West Germany does not pursue Berufsverbote

SIR, — Your note "German psychologists issue study of Berufsverbote" (31 May, page 363) is utterly misleading. The West German government does not pursue a policy of Berufsverbote. Nobody is banned here by the

government to enter any profession of his choice. The federal and state governments reserve the right to select those who apply for employment as civil servants, a status that provides life-long job security. I cannot see anything illiberal when the federal and state governments reject civil service status to a handful of totalitarian radicals. To proceed differently would endanger the liberal system in any western democracy. In my opinion, the slogan Berufsverbote is an infamous lie, cleverly invented by leftist totalitarians to discriminate against the most liberal political system that has ever evolved in the rather sad course of German history.

Yours faithfully,

F. MILLER
University of Munich, West Germany.

Degree-day values

SIR, — In a recent issue of *Nature* (14 June, page 565) the potential value of the 'degree-days' measure was indicated. Readers who wish to obtain degree-day values for the UK may be interested to know that such information is available in *Energy Management*, published by the Department of Energy; and is also reproduced in *Energy Digest*, published by Allens (Clerkenwell) Ltd.

Degree-day users requiring early figures may obtain these from the EQAS service (Freefone 3140, or 8305 in Scotland). Detailed information is available in the free booklet *Degree Days*, available from: Library, Department of Energy, Thames House South, Millbank, London SW1P 4QJ.

Yours faithfully,

P. A. WOOD
Newport Pagnell, Buckinghamshire, UK.

The caddish salamander

SIR, — The vocabulary available to sociobiologists includes altruism, nepotism, selfishness and spite, but is still incomplete. Arnold (*Z. Tierpsychol.* 42, 247; 1976) describes how a male salamander (*Amblystoma*) may intrude between the members of a mating pair, imitate the female, and induce the rival male to release a spermatophore which is then covered with the intruder's own sperm; the latter achieve fertilisation. For this I propose the term *caddish* behaviour.

Yours faithfully,

S.A. BARNETT
Australian National University,
Canberra, Australia.

Erratum

In the issue of *Nature* of 21/28 December, page 754, in a letter by Drs Karlson and Dixon on biochemical nomenclature, the word 'bisphosphate' was twice misprinted as biphosphate in the last two lines of the third paragraph. The authors were pointing out the distinction between 'diphosphate', indicating a single diphosphate group in ADP, and 'bisphosphate', indicating two separate substitutions — as in fructose 1,6-bisphosphate.

news and views

Decline of the calcium hypothesis of visual transduction

from P. Fatt

In a recent issue of *Nature* (Miller & Nicol 280, 64; 1979) and in this issue (Robinson & Hagins, page 398) papers appear which are concerned with the involvement of guanine nucleotides in the action of light on the photoreceptor. In both the authors seem somewhat reluctant to discuss how their findings might fit into an overall mechanism of visual transduction. This reticence is a recently acquired characteristic of workers in the field, for which readers may wish some explanation.

What has happened is that over the period 1972–78 thinking about visual transduction in the vertebrate has been dominated by the ‘calcium-as-the-intracellular-transmitter’ hypothesis, proposed by W.A. Hagins (*A. Rev. Biophys. Bioeng.* 1, 131; 1972) and strongly supported with experimental evidence by him, (see refs in Robinson & Hagins (this issue); Miller & Nicol *Nature op. cit.*) his coworkers and others (see *Vertebrate photoreceptors* (eds Barlow & Fatt) Academic Press, London, 1977). In the case of the retinal rod outer segment, which contains a stacked array of flattened sac-like disks, enveloped by but separated from the surface (or plasma) membrane of the cell, the hypothesis is made up of the following elements. (1) There exists a capacity for pumping Ca ions from the cytoplasmic space (that is, the space internal to the surface membrane but external to the disk) across the disk membrane to the intradiskal space where they are accumulated. This pumping is not directly dependent on the state of the visual pigment (rhodopsin), but is regulated by the concentration of Ca^{2+} in the cytoplasmic space. Operation of the pump requires metabolic energy (which Robinson and Hagins now suggest is provided by hydrolysis of guanosine triphosphate (GTP) to the diphosphate (GDP)). (2) At some stage in the thermal conversion of the visual pigment molecule that follows photoisomerisation of its chromophore (that is, on bleaching), a pore is created in the disk membrane through which Ca^{2+} can penetrate, so that

there occurs a net passage of these ions along their electrochemical gradient, from the intradiskal to the cytoplasmic space. (3) Calcium ions, diffusing about in the cytoplasmic space, act to block pores in the surface membrane which permit the passage of Na^+ between the extracellular and cytoplasmic spaces.

I suggest that a major reason why the hypothesis has now fallen out of favour is the excessive enthusiasm with which it was previously advanced. In its heyday it was offered as the complete explanation of visual transduction. This ignored complications at both (2) and (3) of the hypothetical mechanisms. Measurement of the electrical properties of rods (Falk & Fatt *J. Physiol. Lond.* 299, 195; 1973) gave evidence that pore formation in the disk membrane did not follow directly the bleaching of rhodopsin; the number of rhodopsin molecules that could be engaged in the process over a period of 10 min was limited to 0.05–0.1 of the number initially available for bleaching. Also, the ability to create pores was lost when the surface membrane was damaged, implying that some diffusible material was required. The attribution of a direct role to Ca^{2+} in blocking the Na pores of the surface membrane also had its problems. Ca ion might act either by entering the pore and so clogging it or by combining with a nearby membrane site so as to alter the electric field at the cytoplasmic end of the pore. However, by neither of these means would the Ca^{2+} released from the disks be likely to be as efficacious as quantitative application of the theory required. An indirect mode of action would be less demanding.

In a scheme that has recently come to the fore and is investigated by Miller and Nicol (*Nature op. cit.*) the role of Ca^{2+} is envisaged to end in the activation of the enzyme phosphodiesterase (PDE) which catalyses the destruction of cyclic guanosine monophosphate (cyclic GMP), which is in turn required for activation of another enzyme that leads eventually to opening of Na pores in the surface membrane. Miller and Nicol examine the control of the Na permeability of the surface membrane by experimentally

introduced cyclic GMP and observe the latter's destruction by light-induced activation of PDE. The authors make a passing reference to Ca^{2+} as among the possible activators of PDE.

Robinson and Hagins also give the Ca hypothesis a sideways acknowledgement. This time Ca^{2+} is mentioned as a possible candidate for the transmitter substance, which when pumped back into the disks after its release is considered to account for the observed hydrolysis of GTP to GDP with a time constant of decay of about 10 s following flash illumination. There is also a smaller component of GTP loss attributed to an accelerated turnover of cyclic GMP. As the authors point out, their most unexpected finding is a change in the state of binding of GDP occurring within 4 s of the flash (and possibly within a much shorter period). One may presume this to be related to events taking place in the disks. On the Ca hypothesis the binding of GDP would be involved in the creation of pores in the disk membrane. What is surprising is that the magnitude of the effect is such that some 50 GDP molecules are taken up for each rhodopsin molecule bleached. The question then arises whether all 50 molecules are required to create a single pore or whether the bleaching of a single rhodopsin molecule results in 50 pores, providing chemical amplification.

The latter question leads to the immediate cause of the decline in popularity of the Ca hypothesis. During the past year Liebman and Pugh (*Vision Res.* 19, 375; 1979) have proposed a model of visual transduction in which events between bleaching of rhodopsin and activation of PDE are considered to take place through the diffusion and chemical interaction of these and associated molecules in the disk membrane. An initial stage of chemical amplification is proposed, its gain being given by the ratio of PDE molecules activated to rhodopsin molecules bleached, a number which may well be the same as the factor of 50 found for GDP uptake. In this model there is no role for the pumping of Ca^{2+} or any other transmitter substance across the disk membrane, nor for the creation of pores in the membrane consequent on the bleaching

of rhodopsin. Liebman and Pugh attempted to test the Ca hypothesis and claim to have falsified it by measuring light-induced hydrolysis of cyclic GMP in the absence of free Ca^{2+} and showing in a quantitative argument that the observed rate of hydrolysis is of the correct magnitude to account for visual transduction. On the strict application of the scientific method, this should signal the abandonment of the Ca hypothesis. The numerous experimental findings on the involvement of Ca^{2+} in the response of rods to light, as well as the existence of pumping activity and a light-induced permeability increase of the disk membrane, would have to be relegated to the status of inconsequential side-effects. But science, especially biology, does not always work that way. The reason it does not in the present case is that the system under investigation is unlikely to be as

simple as the model of Liebman and Pugh attempts now to represent it, or as the Ca hypothesis did earlier. There is in addition the disturbing factor that the biological material on which the different studies have been made is not the same. Liebman and Pugh used finely fragmented rods to examine the behaviour of separated disks, whereas studies in support of the Ca hypothesis have often been constrained to use undamaged rods in order to yield results consistent with the hypothesis. Under these circumstances the hypothesis has not been quite abandoned, as the letters of both Miller and Nicol, and Robinson and Hagins show. One may expect that it will continue to fare thus until further investigation either causes it to drop from sight completely or permits its reassertion as its rival encounters difficulties. By then, perhaps, its limitations will be better appreciated. □

New wave of superfluid ^4He

from P. V. E. McClintock

A NEW type of propagating wave mode has been found in thin films of superfluid helium-4. Christened 'fifth sound', its discovery is reported simultaneously by two separate research groups, writing in the same issue of *Physical Review Letters*: by Gary A. Williams, Ralph Rosenbaum and Isadore Rudnick of the University of California at Los Angeles (42, 1282; 1979); and by G.J. Jelatis, J.A. Roth and J.D. Maynard working at Pennsylvania State University (*op. cit.*, 1285).

At least four different types of wave motion were previously known to occur in liquid helium-4 below its superfluid transition temperature at 2.2 K. The reason for this extraordinary richness of the liquid's vibrational characteristics lies, of course, in the fact that it behaves as though composed of two quite separate but freely interpenetrating components, the normal fluid components and the superfluid component. The former has properties much like those of any other liquid whereas the latter, having zero viscosity and carrying no heat, is largely responsible for the well known exotic properties of liquid helium. Depending on the applied experimental conditions, vibrations can travel through the liquid in a number of different ways, each with its own name and characteristic (but temperature dependent) propagation velocity. The two components can move together (so-called first sound) or in antiphase (second sound) or the superfluid can be made to vibrate by itself while the normal fluid remains clamped in position, by placing the liquid helium in a porous medium (fourth sound). The newly

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discovered wave mode is a fairly close relation of third sound, which is a wave on the surface of a thin helium film.

A helium film quickly forms on any surface whose lower part dips into the liquid. It is typically up to 200 Å in thickness, depending on the height above the free surface in the helium bath or on the ambient pressure in the case of unsaturated films, and it can support long wavelength modes rather similar to gravity waves on the surface of a classical fluid, but with the van der Waals attraction to the substrate filling the role normally played by gravity. Because viscous forces clamp the normal fluid component and prevent it from moving parallel to the solid substrate, there will be a tendency for the wave to be accompanied by associated temperature changes: at peaks, where the film is thick, there will be extra superfluid and a lower density of heat-carrying normal fluid, corresponding to a lower temperature; at troughs, where the density of superfluid is depleted, the film will tend to be hotter. Normally, however, an additional mechanism comes into play and effectively irons out all such incipient temperature differences. The film is in thermal equilibrium with the surrounding helium vapour, and so a hot spot on the film results in increased evaporation leading to local cooling, whereas the vapour tends to condense preferentially on to any cold spots, thereby warming them. Thus a wave on the surface of the film is usually a pure thickness mode, not accompanied by any changes in temperature: it is known as third sound.

If, however, the evaporation/condensation phenomenon could somehow be inhibited, the wave would

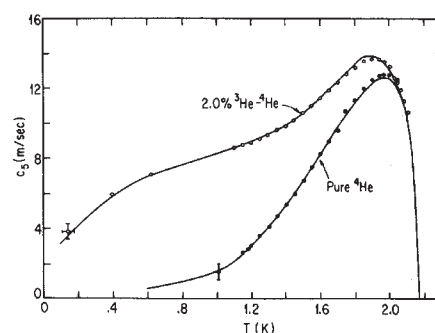


Fig. 1. Experimental values of the fifth sound velocity C_5 (points) compared with the theoretical predictions (full curves) for pure ^4He and a dilute $^3\text{He} - ^4\text{He}$ solution, plotted as a function of temperature (T). (From Williams, Rosenbaum & Rudnick *Phys. Rev. Lett.* 42, 1283; 1979).

then be different in nature and would propagate at a quite different characteristic velocity, because of the additional restoring force arising from the temperature differential between the troughs and peaks. To this end, the Los Angeles and Pennsylvania groups have both resorted to experimental geometries in which the space available for the vapour is carefully restricted. The basic principle is that the separation between the surface of the helium film and the nearest other surface should be made smaller than the mean free path of helium atoms in the vapour. Under such conditions, the vapour is no longer able to transport heat in the direction parallel to the film. Thus the surface wave, instead of being isothermal as in third sound, becomes adiabatic, with temperature minima at the peaks and maxima at the troughs. For relatively thick helium films, where the surface is only weakly influenced by the van der Waals attraction of the substrate, the temperature differential provides the dominant force driving the wave and determining its propagation velocity. It is this thermally driven surface wave which has now been observed for the first time, and which is being called fifth sound.

The two groups used quite different techniques, however, for restricting the vapour. The Los Angeles group worked with standing wave resonances on helium adsorbed on fine alumina powder, compressed so that the average diameter of the pores between the particles was comparable with the mean free path of helium atoms in the vapour. The Pennsylvania group, on the other hand, have succeeded in setting up a more difficult, though conceptually more straightforward, experiment using travelling waves in which a flat plate is placed very close and accurately parallel to the substrate carrying the helium film. They used optically flat quartz plates, and achieved an average spacing of only 5 μm by sandwiching a few grains of powder between them to stop them touching.

In both cases the experimenters found a wave which had a propagation velocity

much faster than that of third sound and, after making due allowance for certain complicating factors, they could deduce the velocity of fifth sound from the measurements. Results from the Los Angeles experiment are shown in Fig. 1. Agreement with the theoretical prediction (lower full curve) is quite remarkably good, and this clearly remains the case even when a little helium-3 is added to change the relative proportions of the normal fluid and superfluid components (the helium-3 contributing only to the former).

Fifth sound constitutes an interesting addition to the rich variety of diverse phenomena already known to occur in liquid helium-4. No doubt efforts will soon be made to observe an equivalent mode in the case of superfluid helium-3: for temperatures around 2 mK the mean free path of vapour atoms is effectively infinite and, to this extent (if not in any other), the experimental design will be simpler. □

Measuring weak absorption spectra

from T.F. Hunter

THE past decade or so has seen a major advance in our ability to measure very weak absorption spectra. Such measurements are needed for analysis of trace amounts of materials or for studying intrinsically weak transitions. In the latter category come vibrational transitions to high energy overtone and combination bands, say in the visible region of the spectrum, and of considerable importance to an understanding of chemical kinetics; spin-forbidden electronic transitions; and transitions due to non-linear coupling of the radiation field with a molecular system, for example, two-photon absorption.

Normal absorption spectroscopy, with its measurement of incident and transmitted intensities, can be of value in this area if sufficiently long pathlengths are used; turntable laser sources, allowing multiple-passage of the radiation beam to be set up more readily, have helped. However, very long optical pathlengths are awkward experimentally, and, in measuring the difference between the incident and transmitted beams, no distinction is made between absorption and scattering, a distinction which may be of consequence for very weakly absorbing samples.

Modern photo-electric detection systems measure the intensity of emitted photons with high sensitivity and, for some time, weak absorptions have been followed by so-called excitation spectroscopy where the intensity of emission is plotted as a function of excitation wavelength. For example, two-photon absorption studies in

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Eukaryotic transcription *in vitro*

by Eleanor Lawrence

IN an unscheduled talk at the recent ICRF Tumour Virus meeting* R. Roeder announced to an appreciative audience the development of an *in vitro* transcription system in which initiation of the adenovirus major late gene transcription unit at the 'correct' site has been unequivocally demonstrated.

He and his colleagues P.A. Weil, D. Luse and Jacqueline Segall at the Washington University School of Medicine, St Louis, have obtained specific initiation and transcription of part of the adenovirus late genes, using either full-length purified adenovirus DNA or restriction fragments containing the start of the transcription unit, in a system consisting of a crude extract (the soluble fraction, S100) of uninfected human KB cells to which purified human RNA polymerase II has been added. Purified polymerases II from other vertebrate cells are also effective but that from wheat germ is not.

Although still in a primitive state this *in vitro* transcription system promises to provide the means by which the basic requirements for the transcription of eukaryotic 'class II' genes can be determined. Class II genes are those transcribed by RNA polymerase II and include many of the single copy sequences in eukaryotic cells, as opposed to the ribosomal RNA genes for example, which are transcribed by RNA polymerase III.

Roeder has yet to test his system on other eukaryotic genes, but the close resemblance of the putative 'promoter' sequences for the adenovirus late genes to those of beta-globin and ovalbumin amongst others, suggests that the system may work equally well for them. Successful transcription of the

adenovirus late genes in the absence of any components from infected cells is of particular interest as the late genes are not transcribed *in vivo* until replication of the adenovirus DNA has begun. This suggests either that these genes are being transcribed at a low level even early in infection, or, more attractively, that control over their expression is exerted at a higher level, that of chromatin organisation perhaps, and that these controls are bypassed when purified DNA is transcribed.

The site at which transcription starts *in vivo* has been mapped precisely (see Ziff & Evans *Cell* 15, 1463; 1978) and so Roeder was able to determine that the 5' end and oligonucleotide fingerprints of the RNA produced in his system matched those of transcripts *in vivo*. Although initiation is specific, transcription does not continue very far into the gene *in vitro* and is also slower than *in vivo*. So one task is to determine whether this premature *in vitro* termination can be overcome, by adding extracts from adenovirus-infected cells for example. The long term aim is gradually to refine the system until it is fully characterised (as with the *in vitro* assays for DNA replication developed for the small bacteriophages). Determination of which regulatory DNA sequences preceding and within eukaryotic genes are essential for faithful transcription is already well underway in the *Xenopus* oocyte transcription/translation system and more recently through the insertion of defined fragments of eukaryotic genes into mammalian cells using SV40 vectors. A fully characterised *in vitro* assay however, could also identify the array of cell proteins that might be expected to be needed.

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*Held on 16-20 July in Cambridge

vapours (Hochstrasse *et al. J. Chem. Phys.* 60, 317; 1974; Schlag *et al. J. Chem. Phys.* 66, 386; 1977) have been studied with high resolution and sensitivity using fluorescence monitoring of this kind. However, many classes of compound show effectively no fluorescence because the dominant deactivation process from the excited state is a non-radiative coupling to other states and other forms of energy.

In any system where the quantum yield of emitted photons is less than unity (and this means essentially all systems), the non-radiative coupling leads to excess heat being produced following the absorption process. An increase in temperature or pressure in the system results. Two techniques, using this flow of heat energy, have been developed.

Thermal lensing spectroscopy (Gordon

et al. J. appl. Phys. 36, 3; 1965; Albrecht *et al. J. Chem. Phys.* 65, 179; 1976) uses a powerful beam of radiation from a tunable dye laser for example. Following the absorption of some radiation by the weak transition under study, the temperature of the sample fluid rises; since the cross-sectional profile of the intensity of the laser beam is Gaussian in shape so is the temperature distribution. Such temperature variation leads to a concomitant variation in the refractive index, a phenomenon which constitutes a thermal lens. The laser beam diverges slightly due to the flow of heat energy. By intensity-modulating the incident beam and thus enabling lock-in devices to minimise interference from noise (thermal, optical, electrical), and by monitoring a part of the transmitted beam through a pin-

hole onto a photo-cell, the resultant signal as a function of the radiation wavelength gives good spectral information on weak transitions. Absorptivities of around 10^{-4} cm^{-1} are readily determined, most measurements having been done on liquids.

The other technique, using the flow of heat energy, is that due to the optoacoustic effect. This effect is of long standing (Bell *Amer. J. Sci.* **20**, 305; 1880) and has been much used, especially in gas-phase studies, for examination of molecular relaxation processes, for example, vibrational-translational energy transfer, de-activation of triplet states and photofragmentation processes (Read *Adv. Mol. Relaxation Processes* **2**, 257; 1967; Hunter *et al. J. Chem. Soc. (Faraday II)*, **70**, 1010; 1974; Hunter *et al. Chem. Phys. Lett.* **58**, 291; 1978). the optoacoustic (OA) effect consists of detecting the oscillatory pressure changes in a system following absorption of intensity-modulated radiation. This is most commonly done in the gas-phase using condenser or electret microphones, and the combination of this technique with laser excitation has enabled the detection of trace gases with high sensitivity, of the order of 1 p.p.b. in many cases (Kreuzer *et al. Science* **173**, 45; 1971; Patel *et al. Appl. Phys. Lett.* **30**, 578; 1977). Absorptivities as low as 10^{-10} cm^{-1} have been measured. Such trace gas detection has normally been done at one, carefully selected, laser wavelength, but obviously, with tunable lasers, weak absorption spectra can be studied. The most sensitive arrangement is to have the OA cell within a dye laser cavity, where a high level of circulating radiation power is present, and high resolution spectra of methane and ammonia in the visible have been so obtained (Stella *et al. Chem. Phys. Lett.* **39**, 146; 1976).

Two recent papers in *Nature* by C.K.N. Patel and A.C. Tam (**280**, 302, 304; 1979) describe important extensions of the OA technique to the measurement of very weak absorptions in liquids. These authors have used as their periodic excitation source a pulsed tunable dye laser, with pulse width of 1 μs and pulse repetition rate at 10 pulses per second, and have developed a very useful pressure transducer based on a lead zirconate titanate cylinder which is submerged in the liquid. Accurate absorption data are given for pure water over the range 400 to 700 nm, and, perhaps more important, details of two-photon absorption in liquid benzene are presented both for linearly and circularly-polarised excitation. The high photon density, both in space and time, required for two-photon studies is achieved by using pulses and by careful focusing of the laser beam; the sample cell is external to the laser cavity.

OA measurements on liquids have been carried out before but in general have used the same technique as that used in the study of solid samples (Rosencwaig *et al. J. appl. Phys.* **47**, 64; 1976; McDonald *et al.*

J. appl. Phys. **49**, 2313; 1978). The surface of the solid, or liquid, is irradiated and the periodic flow of heat is transmitted to a gaseous column containing the microphone. Although widely used in examining solids (several commercial instruments are available) where scattering is a major difficulty with other techniques, the OA method, with a gas as intermediary, has limitations in sensitivity and overall resolution. This is particularly true for solids of high thermal conductivity. The use by Patel and Tam of pressure transducers actually in the liquid, or, for solids, bonded to the surface and thus measuring the sound waves in the solid directly (Farrow *et al. Appl. Opt.* **17**, 1093;

1978), promises to be much more sensitive than the normal approach.

I have concentrated here on the use of the OA technique to measure weak absorptions; its use in the more specialised areas of energy transfer such as photofragmentation shows equal promise. As a detection system it covers all excitation wavelengths, the only requirement being that the excited states produced relax, in whole or in part, to heat energy; thus advances in laser technology (the production of fully tunable infrared lasers) and further improvements in the electronic ability to recover signals from noise will ensure progress in the range and sensitivity available. □

Implications of immunological heterogeneity of tumours

from Robert S. Kerbel

THE concept of 'tumour progression', coined some 25 years ago by Leslie Foulds, has stood the test of time. Its importance cannot be overestimated: to understand tumour progression is to understand why cancer is such a frustratingly difficult disease to treat. Simply stated, tumour progression is the process of independent, stepwise and permanent changes in the many different properties of a malignant tumour. Immunogenic specificity, karyotypic diversity, drug-resistance, hormone-dependence, metastatic potential, degree of cellular differentiation and conversion to ascitic growth are some of the properties which can change as the tumour develops. The process of progression is thought to have its basis in the continuous emergence of successive clones of tumour cell variants, one gradually replacing another, through the intervention of natural or artificial selection pressures.

Progression implies that a tumour cannot be simply a homogeneous population of malignant cells. The undoubted monoclonal origin of most tumours in turn implies an inherent instability (probably genetic, as argued persuasively by Nowell (*Science* **194**, 23; 1976)) which results in tumours continually throwing out mutant cells, a few of which have a selective advantage and become the progenitors of new clones which eventually predominate. The result is the slow but inexorable evolution of an increasingly malignant and autonomous tumour, one not subject to most of the normal constraints imposed by the host.

Immunogenic heterogeneity within a tumour obviously has profound

implications not only for scientists studying the immunological relations between host and tumour but also for the practical application of specific immunotherapy, especially in relation to tumour recurrence and metastasis.

The first indications of immunogenic heterogeneity were provided by Prehn in 1970 (*J. Natn. Cancer Inst.* **45**, 1039; 1970) who established two sublines of cells with different antigenic specificities each derived from different fragments of tissue taken from opposite sides of a primary methylcholanthrene-induced mouse sarcoma. A pair of cell lines established in a similar manner from later serial passages of the same tumours did not differ, however. Prehn concluded that primary or early-passage tumours possess marked, random antigenic heterogeneity which can be lost during subsequent passage as the result of the immune selection exerted against the more immunogenic clone(s).

The importance of such results to the problems of tumour recurrence and metastasis were nicely illustrated by subsequent work performed in many laboratories. It is well known that if cells from established transplantable tumours are inoculated subcutaneously into mice and the resultant tumour removed early enough, the tumour does not regrow at the primary site. The animals also become highly resistant to a later injection of cells from the same tumour line. However, if primary methylcholanthrene rat sarcomas were surgically resected, secondary regrowths almost invariably developed at the resection site (Pimm & Baldwin *Int. J. Cancer* **20**, 37; 1977). Furthermore, cell lines established from such recurrent tumours were found to be immunologically distinct, as assessed by immunisation-protection experiments, from lines established from their original primary

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sarcomas. Similarly, metastases, that is, tumour recurrences found at a site distant from the original tumour, may contain antigens different from those of the primary tumour, or may have lost some antigens completely — the so-called deletion mutants, for example (Sugarbaker & Cohen *Surgery* 72, 155; 1972).

The significance of such immunological heterogeneity should not be lost on those who use established transplantable tumours to study the immunology of the tumour-host relationship. What it means in relation to the specific immunotherapy of experimental tumours is made abundantly clear by some recent experiments reported by Olsson and Ebbesen (*J. natn. Cancer Inst.* 62, 623; 1979). They attempted to prevent the growth of AKR mouse thymic lymphomas (of recent origin), by repeatedly injecting young mice with irradiated AKR thymoma cells, after the recipients had been injected with untreated cells of a primary AKR thymoma. Not surprisingly, the procedure was generally unsuccessful; about 70% of the treated mice developed thymomas. What was surprising was that the thymomas which arose in these mice were immunologically distinct from those in untreated control recipients, as assessed by antibody and cytotoxic T cell assays. After analysing many tumours, the authors concluded that virtually all AKR thymomas are immunologically polyclonal, consisting of a dominant clone, and three distinct minor subclones — the latter group comprising less than 3% of the original tumour cell population. By treating animals with unfractionated thymoma cells, effective immunity is induced only against the dominant clone since the cells of any minor clone are present in too small a number to stimulate the immune system. This merely allows the cells of a minor or 'silent' clone to flourish, so that it eventually becomes dominant. When Olsson and Ebbesen treated their animals with a mixture of equal and large numbers of cells from each clone, so that effective immunity would be produced against all the clones, they produced a cure rate of greater than 90%.

These results also suggest that tumour progression may be more complex than previously thought. It may be that newly emerging clones do not always have to replace one another successively or die off. Rather, many may lead a chronic but cryptic existence, content to live in the shadow of the dominant clone. Only with a favourable change in the immunological *status quo* do they get a chance to flex their muscles.

It would be tempting to blame the failures of specific tumour immunotherapy on immunological heterogeneity and the existence of 'silent' subclones. There are, however, many who would say this is nonsense since most naturally arising tumours are not demonstrably immunogenic (Hewitt *et al. Br. J. Cancer*

Is NGF an enzyme?

NERVE growth factor (NGF) is a protein with a key role in the growth and differentiation of immature sympathetic nerve cells and in the maintenance of fully differentiated sympathetic neurones.¹ Since its discovery in several organs, cell lines and body fluids, biochemists have faced the apparently simple but actually very difficult task of isolating and identifying the polypeptide responsible for such impressive biological action. Attempts to purify this 'molecule' from cell-free extracts have been hampered by the tendency of NGF to associate with other components of the homogenates. Thus the apparent size and sedimentation constant of the NGF 'molecule' have varied according to isolation procedures used. The molecular identity of NGF has changed from a protein of molecular weight 44,000 (ref. 2) to a component of molecular weight 40,000 which is strongly associated with other components of the extract so that together they elute as a protein larger than 100,000 (ref. 3). A form of NGF defined as 7S on the basis of its sedimentation behaviour has also been isolated and found to be⁴ a mixture or complex of three proteins defined as α , β , γ . While γ is an enzyme with arginine esterolytic activity and α has no detectable biological or biochemical action, β is the only component of the complex endowed with nerve growth promoting activity. Subsequent studies suggested that the function of the γ component in an intact cell is to cleave a pro-NGF chain of larger size, as demonstrated for hormones such as insulin⁵.

Finally, a 30,000 molecular weight NGF consisting of two identical polypeptide chains held together by non-covalent bonds was isolated in pure form and defined as 2.5S NGF⁶. The β and

2.5S NGF are almost identical proteins differing only in an N-terminal octapeptide with no detectable relevance to nerve growth promoting activity. This latter preparation has been sequenced and studied extensively⁷. All attempts to detect enzymatic activity in 2.5S NGF have given negative results.

In a recent article NGF has been reported to be a specific protease although the experiments supporting this conclusion leave room for doubt⁸. The authors isolated from the same source as that of the 2.5S or β NGF, the mouse salivary gland, a protein(s) with molecular weight of 116,000. In acrylamide gel electrophoresis in the absence of detergents or other denaturing substances, this protein(s) runs as a single band which exhibits a protease activity with an almost evanescent degree of specificity for plasminogen (880 ng of this preparation have an activity comparable with 0.083 ng of urokinase). The same band cross-reacts with antibodies directed against 2.5S NGF, a finding which is taken as evidence that NGF is a protease. The authors have overlooked the possibility that the apparently homogeneous 116,000 molecular weight component with protease activity, may be a complex of 2.5S or β NGF plus some other protein(s) easily identifiable with the many proteases present in the salivary glands. Their conclusion requires support from evidence obtained in carefully controlled dissociating conditions that the 116,000 molecular weight complex is indeed composed of a single polypeptide chain and is not a mixture of at least two different entities: one with properties of the 2.5S or β NGF but devoid of enzymatic activity and the other(s) with protease activity. The same authors while reporting this enzymatic property in the 116,000 molecular weight protein acknowledge that the 2.5S NGF alone does not possess such activity. Although all previous work indicates that NGF is associated in mouse salivary glands with proteases, this association is not evidence that NGF itself is an enzyme.

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33, 241; 1976). Indeed, the Kleins have stressed the importance of the unrelenting process of tumour progression in the evolution of non-immunogenic spontaneous tumours, whereby successive immunogenic clonal variants replace each other as a result of selection, until a variant emerges which, lacking tumour antigens, becomes independent of the usual restrictions imposed by the immune system (*Proc. natn. Acad. Sci. U.S.A.* 74, 2121; 1977). This remains a sensitive issue, and

the antigenic properties of human tumours are still far from clear. Perhaps the extensive use of hybridomas to produce monoclonal antibodies against putative human tumour-specific antigens will help eventually to resolve this controversy (Herlyn *et al. Proc. natn. Acad. Sci. U.S.A.* 76, 1438; 1979).

The non-immunogenicity of many primary spontaneous tumours and the polyclonal nature of those that are immunogenic, do not auger particularly

well for the future of specific immunotherapy. One possible route to overcoming this problem, as the Kleins have pointed out, is tumour cell modification effected by various artificial means. Another is to concentrate more effort on non-specific immunotherapy. The rationale is fairly simple: a few types of lymphoreticular cell, namely activated macrophages and natural killer (NK) cells, seem capable of spontaneously killing various kinds of tumour cell populations. Because classical antigens are not involved in the recognition process — at least as far as activated macrophages are concerned — variants resistant to this form of effector cell killing may arise far less frequently, if at all. Fidler, for example, has shown that it is possible to derive tumour variant sublines resistant to conventional lymphocyte-mediated killing by progressive selection *in vitro* (Fidler *et al. Cancer Res.* 36, 3160; 1976); the variants appeared to have lost the tumour antigens (Fidler & Bucana *Cancer Res.* 34, 3945; 1977). There are no reports (yet) of the derivation of similar macrophage-resistant (Mac^R) variants. Whether this is from lack of trying is not clear, but if Mac^R tumour variants prove to be rare, it would provide a ray of hope in the fight against the forces of tumour progression. □

Stellar rotation in Erice

from Evry Schatzman

EVEN stars of the most classical types, if they are rotating, are extremely complicated. The convection zones of any star are difficult enough to understand; nevertheless, the problems facing astrophysicists begin to multiply when the stars are rotating. The generation of a magnetic field, for example, is certainly related to rotation — but how?

The workshop* was restricted to the problems of the instabilities associated with rotation and to the effect of rotation on turbulence. The problem of magnetic fields, which is itself formidable, was purposely limited to a few basic questions concerning its relation to turbulence and rotation. On the other hand, getting hydrodynamicists and astrophysicists together was a good way of finding out which kinds of astrophysical problems can be approached by laboratory experiments, and of determining how far we can go in applying present hydrodynamical knowledge to astrophysical problems.

It is naturally the duty of the astrophysicists to list the problems which seem to depend on processes associated with rotation. In other words, what kinds of observations inform us directly about

the physics of the interiors of stars? These observations can be roughly classified according to the depth at which the processes take place, as follows:

- (1) magnetic fields in the filaments at the surface of the Sun (perhaps a few scale heights);
- (2) supergranulation (the depth of the convective zone);
- (3) symmetry properties between the North and South hemispheres of the Sun (flow throughout the whole convective zone);
- (4) lithium depletion by transport from the bottom of the convective zone to the lithium burning level (about one scale height below the convective zone);
- (5) ¹²C/¹³C anomalies in giant stars on the first ascending branch of the Hertzsprung-Russell diagram (region in which ¹³C nuclear processing takes place in main sequence stars, that is, about 1/3 of the stellar radius in from the surface).

In addition, the problem of the origin of white dwarfs' magnetic fields involves the dynamo process in the convective core before large mass loss and white dwarf formation, and consideration of the properties of angular momentum of stars addresses the problem of transfer of angular momentum in the whole star, during formation and on the main sequence.

Only partial answers can be given to these questions. N. Weiss (University of Cambridge) and Galloway (Boulder, Colorado) presented several aspects of the dynamo mechanism, and it is worth stressing that, after years of optimism, the existence of the turbulent dynamo does not seem to be so firmly established. Galloway described the first results of a very large numerical experiment of Gilman (Boulder, Colorado) to simulate the building of the solar-cycle, the significance of which turns out to be very difficult to determine. F. Busse (University of California, Los Angeles) described the role of boundaries on the production of rolls in a rotating thermally unstable region and suggested very strongly that symmetry properties in the solar hemisphere and in the hemispheres of Jupiter can be due to the presence of these rolls.

All the problems of mass transport can be interpreted in terms of meridional circulation or of mixing associated with various instabilities (Rayleigh, shear flow, thermal enhanced). Busse revived the controversy (between Randers, Temesvary, Roxburgh and Baker-Kippenhahn) about the existence of Eddington-Sweet currents by showing that the only stationary solution for a rotating star is a distribution of angular velocities which balances exactly the force induced by stellar oblateness, and cancels the Coriolis force by the suppression of the meridional velocity. S. Temesvary (Tubingen) showed, after Busse, that all non-stationary stream flows decay with the time scale of viscous dissipation, so that the Sun can have any stream flow.

Similarly, for heavier stars (radiative viscosity, electron scattering), the ratio of the viscous dissipation time to the stellar life time grows like M^{-1} . It seems likely that no star lives long enough on the main sequence to reach the magic stationary situation. Therefore, meridional circulation seems to be there. However E. Schatzman (Observatoire de Nice) showed, using Michaud *et al.*'s arguments that, if the meridional circulation is responsible for the lithium depletion in the Sun, this contradicts the fact that there is almost no beryllium depletion. J. P. Zahn (Observatoire de Nice) described the conditions for various shear instabilities and Schatzman showed that a consistent picture can be given, with the same turbulent process, probably related to shear flow instability, which explains a variety of abundance peculiarities for elements nuclearly processed deep inside the stars.

The general properties of stellar rotation on the main sequence are now understood, but the detailed mechanisms remain to be established. Then one would not have to rely merely on phenomenology, as one does in discussions of magnetic field generation.

A discussion on the condition in which angular momentum transport takes place in a turbulent flow showed clearly that this problem is still far from being solved. □

Evry Schatzman is at the Observatoire de Nice



A hundred years ago

The Americans have stolen a march upon us in sanitary matters, as they have done in so many other things. In March of this year an Act passed the US Congress organising a Board of Health to look after all matters relating to the public health, and to advise Congress what steps should be taken for the promotion of so all-important a matter. We have just received the first number of the weekly *National Board of health Bulletin*, published by this body, who have 50,000 dollars appropriated to them for salaries and expenses. Besides mortality statistics the *Bulletin* contains a number of rules and regulations with respect to the sanitary condition of ships.

Under the date of April 30, a correspondent of the *North China Herald* at Wladivostock, in Russian Manchuria, writes that, although the mountain tops were still covered with snow, the winter had at length come to an end, the ice in the harbour having on that day broken up and floated out to sea. The winter began in October and has been the severest known for many years. The bay was entirely covered with ice from two to three feet thick. Wladivostock is said to be improving, though slowly, and there are signs of an attempt at road-making.

From *Nature* 20, 31 July, 32; 1879.

*A workshop on rotating stars was held on 2-10 June in Erice.

review article

Population biology of infectious diseases: Part I

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If the host population is taken to be a dynamic variable (rather than constant, as conventionally assumed), a wider understanding of the population biology of infectious diseases emerges. In this first part of a two-part article, mathematical models are developed, shown to fit data from laboratory experiments, and used to explore the evolutionary relations among transmission parameters. In the second part of the article, to be published in next week's issue, the models are extended to include indirectly transmitted infections, and the general implications for infectious diseases are considered.

ANY contemporary ecology text contains at least one chapter devoted to predator–prey interactions. The discussion typically embraces field and laboratory observations along with simple mathematical models, and emphasizes how the densities of both prey and predator populations may be regulated by their interaction.

In natural communities, however, an accumulating body of evidence suggests that parasites (broadly defined to include viruses, bacteria, protozoans, helminths and arthropods) are likely to play a part analogous, or at least complementary, to that of predators or resource limitation in constraining the growth of plant and animal populations. Examples from the laboratory are Park's¹ experiments in which the sporozoan parasite *Adelina* drastically reduced the population density of the flour beetle *Tribolium castaneum*, and in certain circumstances reversed the outcome of its competition with *T. confusum*, and Lancinani's² studies of the way the ectoparasitic water mite *Hydryphantes tenuabilis* influences the population dynamics of the aquatic insect *Hydrometra myrae*. Various studies have indicated the importance of infectious disease as a mortality factor in populations of wild mammals^{3,4}, and as possibly the predominant such factor in bird populations^{5–7}. For example, among bighorn sheep in North America the main cause of death probably is infection by the lungworms *Protostrongylus stilesi* and *P. rushi*, which then predispose the hosts to pathogens causing pneumonia^{8,9}. On a grand scale, Pearsall¹⁰ and others suggest that the geographical distribution of most artiodactyl species in Africa today is largely set by a pandemic of rinderpest that occurred towards the end of the nineteenth century; the numerical simulations of Hilborn and Sinclair¹¹ confirm that rinderpest can have a big influence on wildebeest population levels. Several authors^{3,5,12–20} have argued the general case for infectious diseases as regulators of their host populations.

More broadly, it is likely that interplay between the pathogenicity of viral, bacterial, protozoan or helminth infections and the nutritional state of the host contributes importantly to the density-dependent regulation of natural populations¹³, with the parasites greatly amplifying the effects of low levels of nutrition. Such phenomena are largely responsible for the dramatic differences between age-specific survival probabilities for

people in developed and underdeveloped countries^{21,22}. Indeed, McNeill²³ and others^{24,25} have speculated that many of the broader patterns of human history are to be interpreted in terms of the evolving relationships between man and his diseases.

Although there does exist a large and mathematically sophisticated literature dealing with the transmission dynamics of parasitic infections of many kinds, this literature^{26–34} almost invariably assumes the host population to have some constant value, and then seeks to answer such questions as: Can the infection be stably maintained in the population? Is it endemic or epidemic? What is the time course (in terms of susceptibles, infectives and recovered individuals) of the infection when introduced into a virgin population? This assumption that the total host population is effectively constant derives from a history of medical interest in human diseases (predominantly in developed countries), where population densities do usually remain roughly constant on the time scale appropriate to the pathology of most diseases. On the other hand, in the ecological and parasitological literature attention has recently been given to the population dynamics of host–parasite associations, with particular emphasis on the way protozoan, helminth and arthropod parasites can depress the natural growth rate of their host populations^{13–17,35}. Our review aims to weave together these medical and ecological strands, concentrating on the way parasitic infections can influence the growth rate of their host populations.

The article is being published in two parts. This first part begins with a survey of the diverse array of infectious organisms and of their associated life cycles. We then show how a very simple dynamic model can provide a remarkably detailed explanation of a classic series of experiments on infections in laboratory populations of mice. This success gives the confidence to enable us to proceed into areas less well supported by good data, and we next discuss microparasitic infections with direct life cycles in natural populations; particular attention is given to the evolutionary relations among transmission parameters, the factors which determine the pattern of disease behaviour within populations of hosts and the population consequences of acquired immunity.

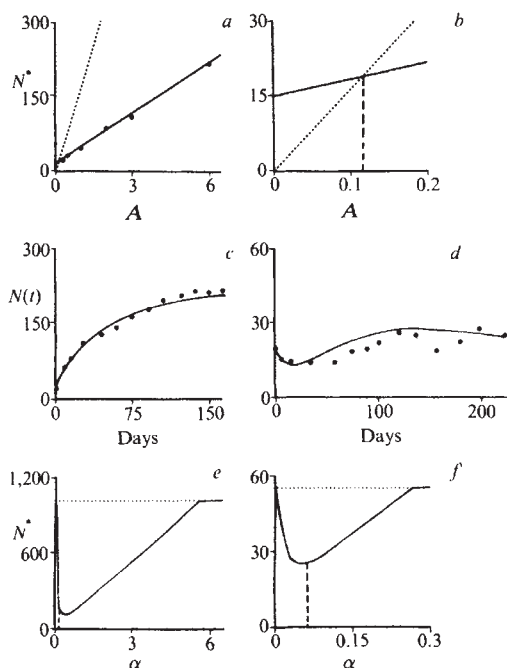


Fig. 1 Population dynamics of *Pasteurella muris* in colonies of laboratory mice. *a*, Relationship between the equilibrium population of mice N^* and the daily rate of input of susceptible mice A (solid dots are observed levels^{46,47}; the solid line is the best linear fit, equation (6)). The dashed line shows the estimated relationship between N^* and A in the absence of the disease (the slope is $1/b$, where $b = 0.006$). *b*, An enlargement of that portion of (*a*) where solid and dashed lines intersect, determining the threshold level of immigration A_T , equation (5), below which the disease will not persist. *c*, *d*, Growth of mouse colonies harbouring the disease, from an initial population of 20 mice, for $A = 6.0$ and 0.33 , respectively (again, solid dots are the experimental data, and the solid lines are the theoretical predictions described in the text). *e*, *f*, Relationship between the equilibrium population of mice N^* and the disease-induced mortality rate α , as predicted by equations (1)–(3), for $A = 6.0$ and 0.33 , respectively. The dotted vertical line shows the actual value of α for *P. muris*.

In the second part of the article³⁶, we begin with a discussion of macroparasitic infections with direct life cycles in natural populations. Extensions to parasites with indirect life cycles are then briefly indicated, with emphasis on the way the ecology of the general evolutionary trends. Finally, we survey the main mechanisms that can produce cyclic patterns, or multiple stable states, in the levels of infection in the host population.

Diversity of agents causing disease

By using the term 'parasitic infection' to include all organisms—viruses, bacteria, protozoans, helminths and arthropods—on the US Centre of Disease Control's list, we are encompassing a great diversity of life forms and of associated population parameters. Broadly, however, two classes may be distinguished:

Microparasites (viruses, bacteria, protozoans) are characterised by small size, short generation times, extremely high rates of direct reproduction within the host, and a tendency to induce immunity to reinfection in those hosts that survive the initial onslaught³⁷. The duration of infection is typically short in relation to the expected lifespan of the host, and therefore is of a transient nature (there are, of course, many exceptions, of which the slow viruses³⁸ are particularly remarkable).

Macroparasites (parasitic helminths and arthropods) tend to have much longer generation times than microparasites, and direct multiplication within the host is either absent or occurs at a low rate. The immune responses elicited by these metazoans generally depend on the number of parasites present in a given host, and tend to be of relatively short duration^{39,40}. Macroparasitic infections therefore tend to be of a persistent nature¹³, with hosts being continually reinfected.

Both microparasites and macroparasites may complete their life cycles by passing from one host to the next either directly or indirectly via one or more intermediate host species. Direct transmission may be by contact between hosts (for example, venereal diseases) or by specialised or unspecialised transmission stages of the parasite that are picked up by inhalation (such as common colds), ingestion (such as pinworm) or penetration of the skin (such as hookworm). Indirect transmission can involve biting by vectors (flies, mosquitos, ticks, and others) that serve as intermediate hosts, or penetration by free-living transmission stages that are produced by molluscan or other intermediate hosts. In other cases, the parasite is ingested when an infected intermediate host is eaten by the predatory or scavenging primary host. A special case of direct transmission arises when the infection is conveyed by a parent to its unborn offspring⁴¹ (egg or embryo), as can occur in syphilis and rubella and for many viral infections of arthropods; this process has been termed 'vertical transmission', in contrast to the variety of 'horizontal transmission' processes discussed above.

The natural historian's main concern is often the recondite biological details that make each parasitic infection unique. In contrast, our aim is to understand the basic similarities and differences in terms of: the number of population variables (and consequent equations) needed for a sensible characterisation of the system; the typical relations among the various rate parameters (such as birth, death and recovery rates, transmission coefficients); and the form of the expressions describing the transmission processes. In the absence of such a unified framework, each disease tends to develop its own arcane literature.

Experimental epidemiology: infectious diseases as regulators of laboratory populations of mice

Although there are relatively few studies of the influence of disease upon the dynamics of laboratory populations^{1,2,42–45}, there is a remarkably detailed body of work of Greenwood *et al.*^{46,47}, subsequently extended by Fenner^{48,49}. These experiments, on laboratory populations of mice infected with various viral and bacterial diseases, have some simplifying features which make them particularly amenable to theoretical analysis. Specifically, the space available to the mice was adjusted to keep the population density constant as absolute levels changed; in addition adult mice were introduced at specified rates, so that the basic process was an immigration–death one (removing the time lags and other complications attendant upon recruiting to the population by natural birth processes). In short, many density-dependent complications are avoided by the design of the experiments.

We now outline a simple model that captures the essentials of these experiments, and discuss its fit to the data for two microparasites: one a bacterium (*Pasteurella muris*); the other a virus (ectromelia, a poxvirus). Both parasites multiply directly within the host and induce a long-lasting immunity to reinfection (mice show some loss of immunity to reinfection by *Pasteurella*, but the immunity to ectromelia seems to be lifelong).

Using notation that will be standard throughout this review, we define the absolute number of susceptible (uninfected), infected and immune mice to be X , Y and Z , respectively. The total number of mice, $N = X + Y + Z$, is not assumed to be some independently-set constant, but is set by the dynamics of the infection. A is defined as the rate at which mice are introduced ($A = 2$ means 2 mice introduced per day), and b the natural mortality rate; in the absence of the disease, the mouse population will equilibrate at around $N^* = A/b$. The infection is a direct one, for which the conventional assumption is that the rate at which mice acquire the infection is proportional to the number of encounters between susceptible and infected mice, being βXY where β is some 'transmission coefficient'. The mortality rate for infected mice is taken to be $b + \alpha$, with α

Table 1 The influence of various types of directly transmitted microparasites on host population growth

Type of disease	Growth characteristic (disease regulates host population if expression is negative)	Threshold host population, for successful introduction of the disease
Horizontal transmission		
No immunity ($\gamma \rightarrow \infty$)	$r - \alpha$	$(\alpha + b + v)/\beta$
Life-long immunity ($\gamma = 0$)	$r[1 + (v/b)] - \alpha$	$(\alpha + b + v)/\beta$
Transient immunity (duration $1/\gamma$)	$r[1 + v/(b + \gamma)] - \alpha$	$(\alpha + b + v)/\beta$
Transient immunity and an incubation (latent) period of duration $1/\sigma$	$r \left[1 + \frac{v}{(b + \gamma)} + \frac{(\alpha + b + v)}{\sigma} \right] - \alpha$	$\frac{(\alpha + b + v)(b + \sigma)}{\beta \sigma}$
Transient immunity and disease eliminates reproduction of infected class	$rv/(b + \gamma) - (b + \alpha)$	$(\alpha + b + v)/\beta$
Transient immunity and disease reduces birth rate of infected class to fa	$r \left[\frac{fa - b}{r} + \frac{v}{(b + \gamma)} \right] - \alpha$	$(\alpha + b + v)/\beta$
Vertical (and horizontal) transmission		
Transient immunity and all births from infected class are also infected	$r[1 + v/(b + \gamma)] - \alpha$	$(\alpha + b + v - a)/\beta$; threshold is zero if $a > \alpha + b + v$
Transient immunity and a fraction f of births from infected class are also infected	$r[1 + v/(b + \gamma)] - \alpha$	$(\alpha + b + v - fa)/\beta$; threshold is zero if $fa > \alpha + b + v$

representing the mortality caused by the disease; there is also a recovery rate v . Recovered mice are initially immune, but this immunity can be lost at a rate γ (for permanent immunity, as for ectromelia, $\gamma = 0$). These assumptions lead to the following equations for the dynamics of the infection:

$$dX/dt = A - bX - \beta XY + \gamma Z \quad (1)$$

$$dY/dt = \beta XY - (b + \alpha + v)Y \quad (2)$$

$$dZ/dt = vY - (\gamma + b)Z \quad (3)$$

Adding all three, the equation for the total population of mice is

$$dN/dt = A - bN - \alpha Y \quad (4)$$

This system of equations (which is similar to that illustrated schematically by Fig. 3) differs from usual epidemiological models in that N is a dynamical variable, rather than some specified constant.

The equations have a stable equilibrium solution with the disease maintained in the population if, and only if,

$$A/b > (\alpha + b + v)/\beta \quad (5)$$

Failing this, the disease dies out, and the population settles to its immigration-death equilibrium value at $N^* = A/b$. If equation (5) is satisfied, the disease persists, and the total population is depressed below this infection-free level to the lower value

$$N^* = \frac{A + D(\alpha + b + v)/\beta}{b + D} \quad (6)$$

Here D is defined for notational convenience as

$$D = \alpha/[1 + v/(b + \gamma)] \quad (7)$$

Note that the important threshold phenomena, which enter directly when N is a specified constant^{26,31-34,50}, appear in a more subtle form when N is itself determined by the dynamics of the disease.

In their experiments on the maintenance of pasteurellosis, *P. muris*, in mouse populations Greenwood *et al.*^{45,46} introduced new mice at rates ranging from $A = 0.33$ to $A = 6$ mice per day. The quantities b , α and v can be crudely estimated from life

tables for uninfected populations, and from case mortality and recovery rates (we get $b \approx 0.006$, $\alpha \approx 0.06$, $v \approx 0.04$ days⁻¹). Direct estimate of the parameters β and γ is more difficult.

Using data from Greenwood *et al.*^{46,47} (and reanalysing to discard the transient initial population values en route to the steady state), we obtain the experimental results shown in Fig. 1a for the equilibrium mouse population N^* as a function of A . These data accord well with the linear relation between N^* and A predicted by equation (6). Furthermore, the parameters β and γ may now be roughly estimated from the fit between the theoretical straight line, equation (6), and the data for N^* versus A (we estimate $\beta \approx 0.0056$, $\gamma \approx 0.021$ days⁻¹).

In Fig. 1a, the dashed line depicts the equilibrium mouse population in the absence of the disease, $N^* = A/b$. The intercept of this line with the linear fit to the data for N^* in the presence of the disease yields the threshold immigration rate, A_T , below which equation (5) is violated and the disease cannot persist; Fig. 1b magnifies this aspect of Fig. 1a. We estimate $A_T \approx 0.11$ mice per day (corresponding to an equilibrium population of about 19 mice). Greenwood *et al.* suggested *P. muris* was always maintained in mice populations, but their lowest introduction rate was $A = 0.33$.

With β and γ determined from Fig. 1a, we now have a parameter-free prediction of the temporal development of the infection for any initial number of mice $N(0)$ and introduction rate A . Two such fits between theory and data are shown in Figs. 1c and 1d, for $A = 6$ and $A = 0.33$, respectively. Note the propensity to damped oscillations at relatively small A values. Bearing in mind the complete absence of adjustable parameters, both the fits are extremely encouraging, and strongly suggest that simple deterministic models can be useful even when the host population is small.

How much does the disease depress the mouse population below the level that would pertain in its absence? This general question is answered in Fig. 1e and f, which shows N^* as a function of disease pathogenicity α , for $A = 6.0$ and $A = 0.33$ respectively. Two significant points emerge.

First, the maximum depression of the host population is achieved by a disease of intermediate pathogenicity⁵¹. Too small an α has little effect on N^* , while too large an α violates equation (5) and makes it impossible for the disease to persist. The dashed vertical lines in Fig. 1e and f show the actual value for α for *P. muris*.

Second, note that the higher the immigration rate A , the greater the degree of depression of the host population (relative

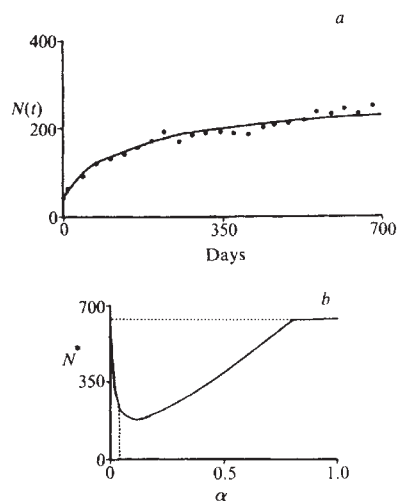


Fig. 2 Population dynamics of ectromelia in colonies of laboratory mice. The data^{46,48,49} indicate $b = 0.005$, $\alpha = 0.042$, $\beta = 0.0013$, $v = 0.014$, $\gamma = 0$; the rate of introduction of susceptible mice was always $A = 3$ (all quantities in units of day⁻¹). a, Growth of a mouse colony harbouring the disease, from an initial population of 45 mice (dots and solid curve as in Fig. 1c, d). b, Depression of the equilibrium population of mice N^* as a function of pathogenicity α for ectromelia, analogous to Fig. 1e, f.

to the disease-free equilibrium value). This suggests that diseases caused by microparasites are more likely to persist within, and cause severe reduction of, host populations with high birth (or immigration) rates; this phenomenon derives essentially from the high inflow of susceptibles.

Greenwood *et al.*⁴⁶, and later Fenner^{48,49}, also studied the effects of the mouse pox virus, ectromelia. An analysis, akin to that just outlined, leads to similarly encouraging agreement between our simple theory and the experimental data for ectromelia in laboratory populations of mice. Some of these results are summarised in Fig. 2.

For both *P. muris* and ectromelia, the actual value of the pathogenicity parameter α (indicated by the dashed vertical lines in Figs 1e, f and 2b) lies around the value that induces maximum depression of the host population. Is this coincidence, or does it reflect evolutionary pressures? The question is intriguing, but difficult to pursue in the absence of a larger body of information about a wider range of diseases.

In brief, the theory and the facts of these experiments are in accord in showing how infectious diseases can stably regulate their host populations below disease-free levels. They also show the existence of a critical host density (directly tied to the rate at which new susceptibles are introduced, either artificially in the laboratory, or by births in the natural world), below which the infection cannot be maintained. In this sense, equation (5) replaces the threshold condition of conventional epidemiological models in which the host population is an independently determined constant.

Microparasitic infections as regulators of natural populations

The models discussed above are only half-way to a fully dynamic description of host-parasite interactions. Although the death rates are set by natural processes, and are influenced by the parasites, the 'birth' processes are determined artificially by the rate of introduction of new mice. We now consider what happens when the birth rates are also set by natural processes intrinsic to the host population.

To begin with, we focus on diseases caused by microparasites that are transmitted directly, and ask three main questions: what biological characteristics of an infection determine its impact on host population growth; what are the population consequences of immunological responses; and what conditions lead to endemic or to epidemic infections?

Consider the simple situation of an infection whose transmission processes are as described by equations (1)–(3), except that now the new individuals arise by natural births. This situation is illustrated schematically in Fig. 3. If the *per capita*

birth rate is a , independent of whether the individual is susceptible, infected or immune, then the net birth rate term is $a(X + Y + Z)$, and the dynamical system of equations (1)–(3) is replaced by

$$dX/dt = a(X + Y + Z) - bX - \beta XY + \gamma Z \quad (8)$$

$$dY/dt = \beta XY - (\alpha + b + v)Y \quad (9)$$

$$dZ/dt = vY - (b + \gamma)Z \quad (10)$$

The total population of hosts, $N = X + Y + Z$, obeys

$$dN/dt = (a - b)N - \alpha Y \quad (11)$$

Equivalently it is useful to define the intrinsic growth rate $r = a - b$ of the disease-free population and to write $y = Y/N$ as the 'prevalence', or fraction of the host population that are infected. This gives

$$dN/dt = (r - \alpha y)N \quad (12)$$

One of two circumstances now arises. If

$$\alpha > r \left[1 + \frac{v}{b + \gamma} \right] \quad (13)$$

the disease regulates the host population to a stable value N^* . This disease-determined population level is

$$N^* = \frac{\alpha(\alpha + b + v)}{\beta[\alpha - r(1 + v/(b + \gamma))]} \quad (14)$$

Of this steady population, the fraction infected is given trivially from equation (12) as

$$y^* = r/\alpha \quad (15)$$

Conversely, if equation (13) is not satisfied, the system of equations (8)–(10) eventually settles to a state in which the total population grows exponentially at a rate ρ given by

$$\rho = [B^2 - (b + \gamma)(\alpha - r) + rv]^{1/2} - B \quad (16)$$

with $B = \frac{1}{2}(\alpha + b + v + \gamma - r)$. This population growth rate is necessarily less than the disease-free one, $\rho < r$. Asymptotically, the exponentially growing total population contains a constant number of susceptibles X , with essentially all individuals being infected or immune. The asymptotic prevalence of infection is

$$y \equiv Y/N \rightarrow (r - \rho)/\alpha \quad (17)$$

Note the similarities and differences between these conclusions and those for conventional models^{26,31–50} in which the total population is set at some constant value. In this crude model there are no density-dependent regulatory effects other than the

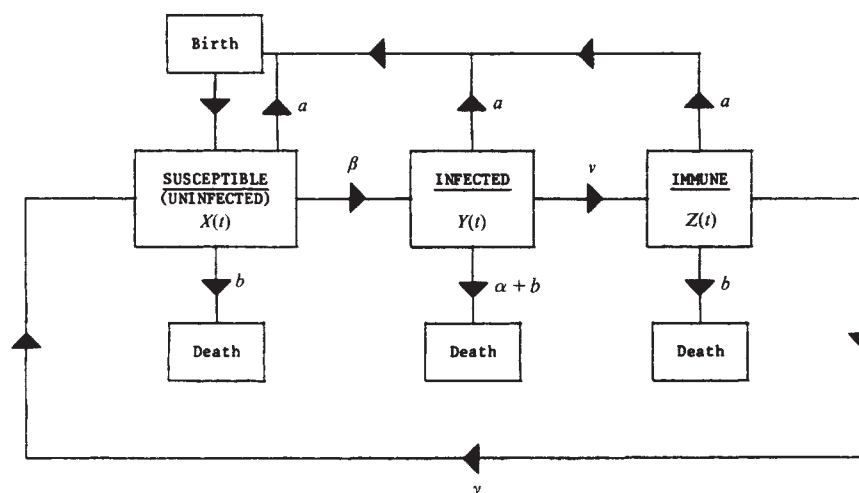


Fig. 3 Diagrammatic flow chart for a directly transmitted infection, described by a compartment model with susceptible (X), infected (Y), and immune (Z) hosts. The flow of individual hosts between compartments is controlled by a set of rate parameters: *per capita* birth rate, a ; natural death rate of hosts, b ; disease-induced mortality, α , acting on infected hosts; recovery rate, v ; transmission rate per encounter between susceptible and infected hosts, β ; rate of loss of immunity, γ .

Table 2 Population characteristics of some common directly transmitted microparasites of man (data from refs 66–69)

Parasite	Incubation period (days)	Duration of infectiousness (communicability) (days)	Infectiousness	Duration of immunity	Lifespan of infective stage	Case mortality rate (pathogenicity)	Transmission (H = horizontal V = vertical)
Measles virus	9–12	5–7	High	Lifelong	Very short	Low–high	H
Smallpox virus	12–14	10	Medium	Lifelong	Long	High	H
Rubella virus	17–20	14	Medium	Lifelong	Very short	Low	H, V
Mumps virus	10–20	7	Medium	Lifelong	Short	Low	H
<i>Bordetella pertussis</i> (whooping cough)	7–10	14+	High	Lifelong	Very short	Medium	H
Polio virus	5–20	Long	High	Lifelong	Medium	Medium	H
Varicella zoster virus (chicken pox and shingles)	13–17	20–30	High	Lifelong	Very short	Low	H (V)
Herpes simplex virus	5–8	Long	Medium	Lifelong	Very short	Very low	H, V
Cytomegalovirus	Long?	Long	Medium	Lifelong	Very short	Very low?	H, V
Epstein–Barr virus	10?	Long?	Medium	Lifelong	Very short	Very low?	H
<i>Clostridium tetani</i> (tetanus)	7+	21–30	Low	Lifelong	Long	High	H
<i>Salmonella typhi</i> (typhoid)	10–14	30+	Low	Short	Medium	High	H
<i>Bacillus anthracis</i> (anthrax)	3–??	?	Low	Long	Very long	Very high	H
<i>Corynebacterium diphtheriae</i> (diphtheria)	2–6	20	Medium	Long	Medium	High	H

disease itself and the population 'runs away' (maintaining the disease within it) at the diminished rate ρ if α is too small to satisfy equation (13). Conversely, if equation (13) is fulfilled, the population settles to the value N given by equation (14). In either case, if N is initially less than a threshold value,

$$N_T = (\alpha + b + v)/\beta \quad (18)$$

then initially Y will decrease and X will increase exponentially at the rate r . However, once X exceeds N_T on this trajectory of exponential increase, then Y will increase, and the system either will converge (steadily or with damped oscillations) on the N^* of equation (14), or will grow at the slower rate ρ of equation (16). Thus the familiar threshold phenomena are found within the more dynamic system of equations (8)–(10).

Equation (13) is clearly a key one. It can be modified to take into account the known biology of a wide range of directly transmitted microparasitic infections. Without discussing the derivations, Table 1 lists the criterion for ability to regulate the host population (generalising equation (13)), and the threshold expression (generalising equation (18)), for a variety of such refinements, including *inter alia* the effects of incubation periods, vertical transmission, and infections that reduce host reproduction.

Several general points emerge from Table 1. (1) For a disease to regulate the host population, the case mortality rate α must be high relative to the intrinsic growth rate r of the disease-free host population. Ability to achieve this degree of regulation is decreased by lasting immunity (γ small) and high rates of recovery from infection (v large, corresponding to infections of short duration). (2) Diseases with long incubation periods, where hosts are infected but not infectious, have less impact on population growth. (3) Diseases which affect the reproductive capacities of infected hosts are more liable to suppress population growth. (4) Vertical transmission lowers the magnitude of the threshold population, N_T needed for successful introduction of the disease; vertical transmission also lowers the equilibrium population of the host in those cases where it is regulated by the disease. (5) The threshold density below which the disease cannot persist within the host population is set by the rate of loss of hosts from the infected class divided by the rate of transmission; high threshold densities are therefore required for the maintenance of diseases with short durations of infection, long incubation periods and high case mortality rates.

Population consequences of immune responses

Although the nature of immunological responses by individual hosts to specific pathogens has received much attention in recent years^{37,40,52}, relatively little thought has been given to the population consequences of acquired immunity^{26,32,53} (sometimes called 'herd immunity' effects). The general insights just culled

from equations (13)–(18) can be usefully illuminated by a numerical example. Figure 4a shows the growth of a fictitious human population (from an initial size of 50,000) subject to a virus disease and under various assumptions about the duration of immunity. The vital rates and transmission parameter values are as detailed in the figure caption. In more homely terms, they represent a growth rate of the disease-free population of around 3% per annum, a case mortality of about 30% (similar to measles in malnourished human populations with no previous exposure⁵⁴), duration of the infection around 4 weeks, and a transmission coefficient β that implies a threshold population density of $N_T \approx 380,000$ people.

In all cases in Fig. 4a, the disease is not maintained and the population grows at its intrinsic 3% rate if it is below the threshold value N_T . Above this point, the population's fate depends on the nature of the immune response. If the duration of the immunity to reinfection ($1/\gamma$) is of short to medium length (less than about 20 yr), the disease is able to regulate the host population at the stable level N^* of equation (14). If the disease induces hardly any immunity (γ large), this equilibrium level N^* will be close to the threshold N_T for maintenance of the disease. Conversely, if the duration of immunity is above 20 yr, the population continues to grow exponentially at some rate lower than 3%; life-long immunity ($\gamma = 0$, as for measles) results asymptotically in 1.6% per annum growth. This example makes plain the important part immunity plays in determining the population consequences of a disease.

The qualitative patterns revealed in Fig. 4a are reminiscent of those shown by human population growth^{55,56} between the beginning of the Agricultural Revolution (some 10,000 years ago) and the onset of the Industrial-Scientific Revolution (around 300 years ago). In the first 5,000 yr, the global population increased about 20-fold, from around 5 million to around 100 million. The next 5,000 yr saw only a roughly 5-fold increase to around 500 million in the sixteenth century. It may not be unduly fanciful to speculate that the rise of human conglomerations to levels capable of maintaining directly transmitted microparasitic diseases, and the accompanying depression of population growth rates, is at least partly responsible for the observed patterns.

Epidemic and endemic patterns of diseases

Epidemic diseases are characterised by rapid changes in the prevalence of infection. Often such infections disappear from a particular host population for short or long periods. Conversely, endemic infections persist for long times, showing relatively little fluctuation in prevalence. Note that in our dynamic models, equations (8)–(10), the disease always becomes endemic, in the sense that the host population grows to the level $N > N_T$, whereupon the disease is maintained. The prevalence settles to the steady value $y^* = r/\alpha$ if the disease controls the population, and to $y^* \rightarrow (r - \rho)/\alpha$ if the population still grows.

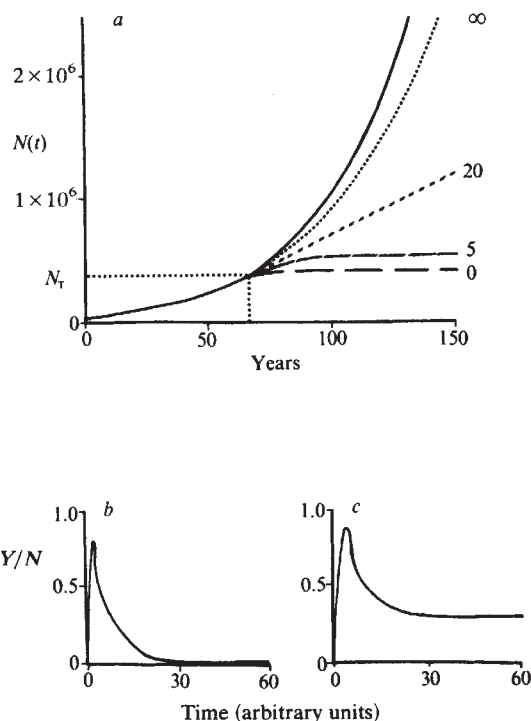


Fig. 4 *a* An illustration of the way the duration of immunity influences the dynamics of host population growth, $N(t)$, for a hypothetical disease; the various population parameters are assumed to be $r = 0.03$, $b = 0.015$, $v = 13.0$, $\alpha = 6.0$, $\beta = 5 \times 10^{-5}$ (all per year), and $N(0) = 50,000$. The solid line depicts the population growth in the absence of the infection. The four broken lines depict the effects of immunity of varying duration, namely (as labelled): $1/\gamma = \infty$ (lifelong); $1/\gamma = 20$ yr; $1/\gamma = 5$ yr; and $1/\gamma = 0$ (no immunity). *b*, Temporal changes in the prevalence of infection, Y/N , following the introduction of the above disease into a virgin population of hosts where the equilibrium prevalence level is low. *c* As for (*b*), except now the equilibrium prevalence is relatively high.

It is well known, however, that diseases which induce long-lasting immunity often exhibit periodic or episodic 'face out', even within relatively large host populations^{26,30,57-61}. In particular, the classic work of Bartlett^{62,63} on measles epidemics has suggested the importance of stochastic effects in determining whether a disease will persist endemically or as recurrent epidemics.

Without entering into the detailed complications of a stochastic formulation, we can use the above model to get some qualitative insights about these patterns. Of particular importance is the rate at which new susceptibles appear; hence the general correlation between endemicity and host population size⁶⁴, and the observation that the host birth rate is central. Specifically, consider the case where the hosts' intrinsic growth rate is much smaller than the case mortality rate, $r \ll \alpha$. Then, if $N > N_t$, introduction of the infection results in a classical epidemic (see Fig. 4*b*): the prevalence first rises, attains a peak, and then falls to the value given by equation (15) or equation (17), which in either case is very small. That is, if $r \ll \alpha$, it is likely that

prevalence settles to be so small as to give a high probability for stochastic 'fade out' and epidemicity. This can be true even for diseases potentially capable of regulating the host population, if r/α and N^* are both sufficiently small. In addition, epidemics can occur even when $r > \alpha$ if the disease does not regulate the host population but merely slows its growth rate slightly, so that $r - \rho \ll \alpha$ (the details of the interplay among parameters that leads to $\alpha + \rho \gg r > \alpha$ are complicated, and can be deduced from equation (16) for ρ). On the other hand, if neither r nor $r - \rho$ is a lot smaller than α , the disease is likely to be endemic, with relatively high values of y^* making stochastic extinction of the disease improbable^{62,63,65,66}. This circumstance is depicted in Fig. 4*c*.

Thus infections of short duration which induce lasting immunity will tend to exhibit epidemic patterns. The classic 'epidemic' disease such as measles, rubella and pertussis are of this character⁶⁷⁻⁷⁰. As also stressed by Yorke *et al.*³², a broader examination of viral and bacterial infections of man clearly supports this point (see Table 2). Many authors^{57,58,71} have observed that such infections are probably diseases of modern societies; in primitive societies the net inflow of susceptibles into small communities was probably too low to maintain the diseases.

Other infectious agents (for example, herpes simplex virus, cytomegalovirus, Epstein-Barr virus) persist in the host for long periods and are of low pathogenicity. Such diseases are usually endemic in character⁵⁷ (see Table 2). Moving beyond human populations, it is important to remember that hosts with high rates of reproduction, such as arthropods, may be able to support endemic disease even if host density is low⁷². Furthermore, infectious organisms that induce life-long immunity, or are of high pathogenicity, can be endemic if they produce free-living infective stages which can survive for a long time in the external environment (anthrax bacillus is an example).

There is no doubt that microparasitic infections can slow population growth⁷³⁻⁷⁵. Whether a given disease will regulate the host population or merely slow its growth, and whether the infection will be endemic or epidemic, depends on the interplay of many biological parameters^{32,70}. Unfortunately, our quantitative knowledge of these parameters is limited, even for viral and bacterial diseases of man.

Conclusion

The effects of microparasitic infections on the dynamics of animal populations depend on the ecology of the interactions between host and parasite. These patterns of disease behaviour involve four principal factors, namely: the host providing a habitat for the parasite; the degree to which the parasite induces host mortality (or diminishes the reproductive capability of the host); the extent to which the host acquires immunity; and the necessity of transmission from one host to the next. Overlaid on these factors are many biological complications, specific to individual host-parasite associations, whose sequential action is determined by life cycle structure.

In the second part of this article, we show how a common set of factors are involved in the dynamics of all infectious diseases, whether they are caused by viral or helminth agents, and whether they are transmitted directly or indirectly between hosts.

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articles

Possible optical observation of the companion star to the binary pulsar PSR1913+16

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Deep R band CCD photographs of the region surrounding the binary pulsar PSR1913+16 have been obtained with the 4-m telescope of the Kitt Peak National Observatory. Using an accurate astrometric solution, we find a star with $M_R = 20.9$ at the precise coordinates of the pulsar. Possible interpretations of this result, including the unlikely (<3%) possibility that it is an accidental superposition of a field star, are discussed. If this object is the physical companion to the pulsar, it is probably a helium star.

THE discovery of the binary pulsar PSR1913+16¹ has created substantial interest as a system in which various general relativistic effects^{2–4} may be measured. The ability to perform these experiments relies critically on two factors: (1) that the pulsar

clock should behave in a regular way and (2) that the companion star must be treatable as a point mass. The former point seems to be satisfied⁵ so the major remaining issue for tests of relativity to proceed is how to determine the nature of the companion star. Since the optical extinction is probably modest ($A_V = 3.3$; ref. 6) we expect that a non-compact companion should be observable with a sensitive detector on a large telescope. Thus, sensitive observations could establish valuable constraints on the size of the companion star. The small observed apsidal motion in the system already limits the companion star to being either a helium star or a compact object³.

The observations

We have used the JPL CCD array (400×400 pixels; 0.45" per pixel) at the prime focus of the 4-m Mayall telescope to observe the region of the binary pulsar PSR1913+16. Our

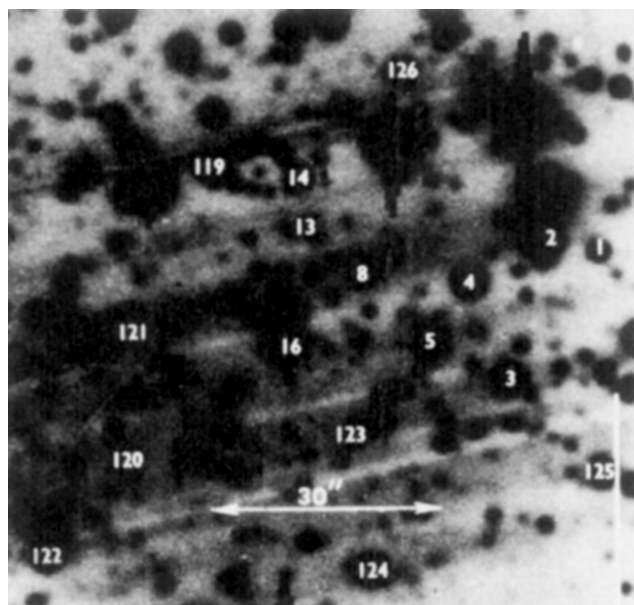


Fig. 1 Finding chart for all the transfer stars on the CCD field (raw data frame). The positions of each star are listed in Table 1. North is up, and east is left. The faint stars 7 and 9 in Fig. 2 are just south of star 8 in this figure.

observational sensitivity would allow us to detect an object with $M_R \leq 23.7$. Within our measurement error of $0.32''$ we find an object with $M_R = 20.9$ at the precise pulsar radio coordinates given by Taylor *et al.*⁷. This is the same object identified by Kristian and Westphal⁸ (also J. Kristian, personal communication) with their positional accuracy of $\approx 1''$.

The region of the pulsar was observed on the nights of 8 and 9 May 1978 and was made with an R filter of the system described by Bessel⁹. This passband is a compromise between going as red as possible to reduce the galactic absorption, while avoiding bright variable red OH features in the sky which cause problems with reduction as well as degrade the intrinsic signal-to-noise ratio.

Figure 1 shows a reproduction of a portion of a CCD frame of the area of PSR1913+16. This is a raw data frame and has not been flattened to remove the variations in sensitivity. Also shown are the 17 transfer astrometric stars which were used to determine the position of the very faint objects in the CCD frame. This is one of a set of three 16-min exposures of this field. Figure 2 shows a more detailed reproduction of the CCD frame which has been processed to remove background variations. The faintest objects visible (such as object 22) have $M_R = 23.8$, or are about 3 mag fainter than the limit of Shao and Liller¹⁰.

The astrometric solution has been obtained using the Palomar Sky Survey plates and the SAO star catalogue. This was done on the IPPS and Grant engine at KPNO, and independently at ESO. Our solution differs significantly from that of Shao and Liller¹⁰. On Kristian's (personal communication) suggestion we performed independent astrometry on copies of the Palomar Sky Survey plates. We put objects 1.6 arc s further east than Shao and Liller¹⁰ and with this solution, we found a star (object 21 in Fig. 2) located within $0.05''$ of the pulsar radio coordinates. The radio coordinate uncertainties given by Taylor *et al.*⁷ are $0.04''$. The positions of 25 objects in the CCD field are listed in Table 1 where the objects numbered <20 correspond to those of Shao and Liller, and the letters correspond to the objects of Kristian *et al.*¹¹.

We now briefly consider the uncertainties in our stellar position. The Palomar Sky Survey plate was measured and fit with a 6 parameter linear function using 29 SAO stars. The scatter in the fit showed an r.m.s. uncertainty of $0.54''$ in each star's position, resulting in an expected r.m.s. uncertainty of $0.11''$ for a

stellar position determined by this solution. This may be due to SAO star coordinate errors or PSS plate distortion or measurement error. Since the CCD picture contains no SAO stars, secondary astrometric standards are needed to establish the CCD picture coordinates. The SAO coordinate grid was transferred to the CCD picture using 17 stars common to both pictures. These transfer stars are numbered in Fig. 1. This transfer process introduced an estimated error $\leq 0.1''$. The overall coordinate grid error in the CCD picture then becomes $0.15''$ r.m.s. The measurement uncertainty of star 21 found experimentally by measuring two CCD frames is $\leq 0.2''$. To check for systematic errors we constructed a coordinate grid using the AGK3 stars and found the errors between the SAO and AGK3 solutions to be $0.2''$. Thus we expect the accumulated error (systematic and statistical) of our coordinate solution for star 21 to be $\leq 0.32''$ r.m.s.

Using a standard star (Kopf 27) observed in the R band with the CCD detector, we have reduced our measurements to spectrophotometric R magnitudes for several stars near the pulsar position. These are also given in Table 1. Our R magnitudes are close (± 0.2 mag) to the I magnitudes given by Shao and Liller¹⁰. For the candidate star we find $M_R = 20.9$.

Interpretation

Within the tight limits set by the accuracy of the pulsar coordinates and our astrometry, several interpretations of our results are possible: (1) the light seen comes from the pulsar itself; (2) a third star in the system produces the light; (3) a field star accidentally lies along our line of sight; (4) the companion star is the light source. We will now consider these possibilities.

(1) If the light comes from the pulsar, one might expect it to be strongly modulated at the pulsar frequency of 16.9 Hz. However, the observations of Nather *et al.*¹² show the upper limit on optical pulsations is $M_R \approx 26.2$. This would imply the pulsed fraction of PSR1913+16 must be $\leq 1\%$. As the two

Table 1 Positions and magnitudes of objects in the vicinity of PSR1913+16

Star	RA (1950.0)		Dec (1950.0)		Magnitude (R)
1	19 h 13 min	10.32 s	+16° 01'	17.6"	
2		10.80		19.1	
3		11.10		01.3	
4		11.44		13.6	
5		11.79		06.4	
8 (H)		12.36		14.6	
13		12.86		20.6	
14 (J)		12.92		26.9	
16		12.96		05.6	
21		12.47	01 08.0		20.9
22		13.03	00 59.1		23.8
23		12.62	01 16.6		20.5
24		13.04	15.7		20.2
25		13.27	15.5		19.6
26		12.42	03.9		21.5
27		12.61	02.4		21.6
28		12.80	00 59.5		22.8
119		13.69	01 28.2		
120		14.39	00 50.9		
121		14.33	01 06.8		
122		15.05	00 38.6		
123		12.43	54.1		
124 (F)		12.28	36.6		
125		10.28	00 49.2		
126		12.04	01 40.4		
Pulsar radio position*		12.474	01 08.02		

Note that stars numbered less than 20 correspond to those of Shao and Liller¹⁰ and letters correspond to Kristian *et al.*¹¹. Star 21 is the candidate originally suggested by Kristian and Westphal⁸.

* From ref. 7.

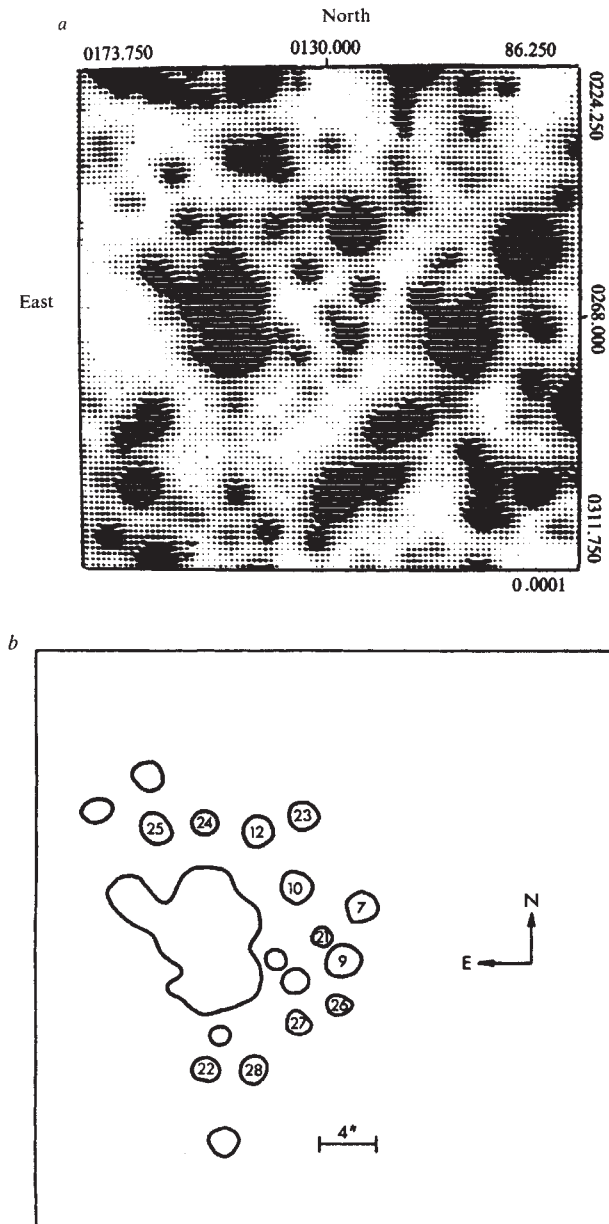


Fig. 2 *a*, Reproduction of the CCD data processed for removal of fixed pattern noise, in the region of the binary pulsar PSR1913+16. Star 21 is the object within 0.05 arc s of the pulsar position. We find a 97% probability that this star is a real association, based on star counts in the field and our measurement uncertainties. Star 22 is the object used as a reference for the limiting magnitude. Other stars correspond to those of Shao and Liller¹⁰. The positions and magnitudes are listed in Table 1. *b*, Identification chart for faint stars of Table 1 and *a*.

known optical pulsars, the Crab and Vela pulsars¹³, show pulsed fractions of ~100% and 50% respectively, this upper limit makes this interpretation unlikely.

(2) If a third star is in the system, it must have survived at least one supernova in the system without being ejected, and must also have produced sufficiently subtle effects on the orbit of the pulsar as to remain undetected until now. This possibility seems extremely unlikely.

(3) To evaluate the possibility that we are seeing a field star, we must consider our measurement uncertainties and the density of field stars. A star would be a valid candidate if that object lay within our error circle 90% of the time. Assuming gaussian errors, this sets the error circle radius as $1.72 \theta_{\text{r.m.s.}}$,

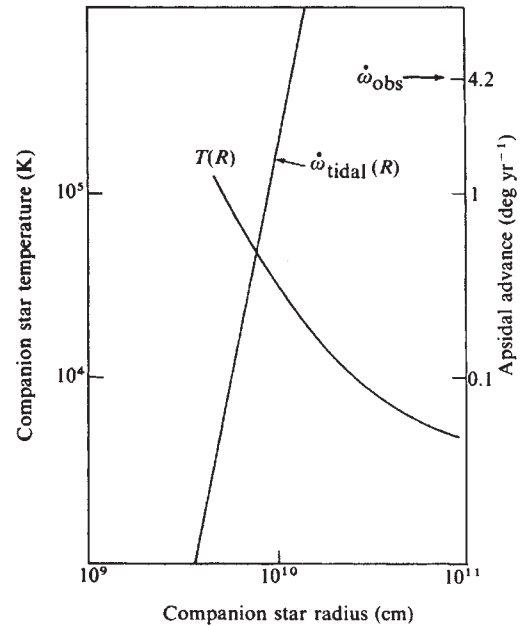


Fig. 3 The relationship between the companion star's radius (bottom scale) and its temperature (left vertical scale) is shown. It is based on our observed flux from the candidate star, an assumed distance and extinction (see text) and the assumption that it radiates as a black body. The apsidal advance (right vertical scale) due to tidal effects is also shown as a function of the companion star radius. The structure of a helium star was assumed (see text). The total observed apsidal advance is also indicated. Simply requiring that the tidal effect be $\leq \omega_{\text{obs}}$ implies $T_c \geq 23,000$ K.

where $\theta_{\text{r.m.s.}}$ is the r.m.s. radial uncertainty due to all statistical and systematic effects. In this case, $\theta_{\text{r.m.s.}} \leq 0.32''$ so the 90% error circle radius is $0.55''$. Since our observed coordinate difference is $\leq 0.05''$, we (formally) accept the candidate star. Using the CCD picture, we have established the density of stars with $M_R \leq 20.9$ at 0.037 stars per arc s², so there is a 3% chance that the star is an accidental association and a 97% chance that it is a real association.

(4) The most likely result is that we have detected the companion star of PSR1913+16. The small observed apsidal motion in the system of $\dot{\omega} = 4.24^\circ \text{ yr}^{-1}$ prohibits hydrogen main sequence stars, since their tidal effects would cause $\dot{\omega} > \dot{\omega}_{\text{obs}}$. Only helium stars and degenerate objects are small enough to be allowed (see ref. 4 and refs therein). We will now estimate some properties of this companion star.

If we assume a distance¹ of 5 kpc and an extinction⁶ of $A_V = 3.3$ and further assume the object radiates as a black body, we can establish a relationship between the radius of the object and its temperature using our observed R flux at $0.68 \mu\text{m}$ of $f_\nu = 1.58 \times 10^{-28} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$ as shown in Fig. 3. This relation implies unrealistically high temperatures for a compact object. If $r = 10^9 \text{ cm}$ (a typical white dwarf) we find a temperature of $2 \times 10^6 \text{ K}$. If the object is a neutron star ($r = 10^6 \text{ cm}$) we find $T = 2 \times 10^{12} \text{ K}$. Thus, if the object is a thermal radiator, it cannot be degenerate and is expected to be a helium star.

A non-compact companion star will, in general, give a tidal contribution to the apsidal motion of the orbit given by (ref. 3, equation (3.8))

$$\dot{\omega}_{\text{tidal}} = 11.2 \left(\frac{k_2}{0.01} \right) \left(\frac{M_p}{M_c} \right) \left(\frac{M_p + M_c}{M_\odot} \right)^{-5/3} \left(\frac{R_c}{0.2 R_\odot} \right)^5 \text{ deg yr}^{-1}$$

in addition to the general relativistic contribution (ref. 3, equation (3.5))

$$\dot{\omega}_{\text{GR}} = 2.11 \left(\frac{M_p + M_c}{M_\odot} \right)^{2/3} \text{ deg yr}^{-1}$$

where M_p and M_c are the masses of the pulsar and its companion, R_c is the companion radius, and k_2 is a structure function measuring the degree of central condensation of the companion star. Roberts, Masters and Arnett⁴ have calculated, using stellar models, the value of k_2 for various helium stars and find $k_2 \approx 0.05$. The apsidal motion due to tidal effects is shown in Fig. 3, assuming $M_p = M_c = 1.4M_\odot$ and $k_2 = 0.05$. Note that for a given apsidal advance, the radius is quite insensitive to the assumed value of k_2 and the masses. Simply requiring that $\dot{\omega}_{\text{tidal}} \leq \dot{\omega}_{\text{observed}}$ means that R_c must be $\leq 1.2 \times 10^{10}$ cm. This in turn implies that the companion star has a surface temperature $T \geq 23,000$ K using the above black-body result. Note that, at least for more massive helium stars, such high temperatures are expected¹⁴. If we include the relativistic apsidal motion then, $\dot{\omega}_{\text{tidal}} = \dot{\omega}_{\text{observed}} - \dot{\omega}_{\text{GR}}$, so R_c must be smaller, and T larger than given above. Only by assuming that $\dot{\omega}_{\text{tidal}} = 0$ does one obtain the system mass of $2.85M_\odot$ needed to account entirely for the $\dot{\omega}_{\text{observed}}$ by the relativistic effect.

Expected colour of the object

Using the reddening relation described by Nandy *et al.*¹⁵ and the extinction of Davidsen *et al.*⁶ we expect $E(V-R) \approx 1.0$. Johnson¹⁶ gives $V-R$ for stars in the above temperature region of ≈ -0.1 . To convert our spectrophotometric magnitude to a Johnson R magnitude we must add -0.2 . We thus roughly expect $V \approx 21.6$. Kristian and Westphal⁸ reported $V = 22.5$. If the given V and R are correct and the object is the companion star, this result suggests that the extinction is greater than that assumed and the temperature of the companion star is hotter.

Conclusion

We have observed a likely candidate (97% probability) for the companion star of PSR1913 + 16. This result could be confirmed by spectroscopic observations. If confirmed, this system might not be a suitable testing ground of general relativity and would bring into question the claimed evidence for gravitational radiation by Taylor *et al.*⁷. The existence of a helium star for the binary companion would also be interesting on evolutionary grounds.

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A κ -immunoglobulin gene is formed by site-specific recombination without further somatic mutation

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The active gene for a κ light chain is formed by a somatic recombination event that joins one of several hundred variable region genes to one of a series of recombination sites (J-segments) encoded close to the κ constant region gene. The nucleotide sequences of cloned germ line and somatically recombined genes define the precise organisation of these genetic segments and the site and nature of the recombination event that joined them. Apart from somatic recombination, no further alteration of the germ line sequence has occurred. The J-segment is of special interest as it encodes signals for both DNA and RNA splicing and provides a means of generating further immunoglobulin gene diversity.

A COMPLETE explanation of antibody gene expression must take into account at least four critical observations—the striking variable/constant structure of antibody molecules, the diversity of their sequences, the fact that only one member of an allelic pair of antibody genes is expressed in a given lymphocyte, and the curious process by which a given lymphocyte seems to be able to sequentially associate a single variable region with two or more different classes of heavy chain constant regions (see

complete bibliography in ref. 1). The argument advanced by Dreyer and Bennett², namely, that variable and constant regions are separately encoded in the germ line and are probably drawn together by somatic recombination, is likely to be relevant to each of these features of antibody gene expression.

Most recently, a series of experiments involving the analysis of DNA fragments encoding variable and constant region immunoglobulin genes has convincingly borne out the predictions of the Dreyer–Bennett hypothesis^{3–5}. In particular, an examination of cloned DNA fragments has added considerable and unexpected detail to our understanding of the arrangement of genes corresponding to immunoglobulin λ and κ light chains^{1,6–9}. Our own experiments have concerned the κ light chain genes of the mouse^{1,4,8}. (Antibody diversity in the mouse depends almost entirely on κ , as opposed to λ , light chains¹⁰.) By cloning individual genes and using *in situ* hybridisation of DNA restriction fragments, we have shown that a large repertoire of κ variable region genes is encoded in the germ line genome. Saturation hybridisation experiments by Valbuena *et al.*¹¹ support this view. In addition, those genes that we have sequenced seem to be foreshortened, that is, they fall 13 amino acid codons short of the conventionally defined variable region sequence⁴. In the case of the MOPC-41 κ chain, we were able to show that formation of the active gene involved a recombination event that joined such a foreshortened variable region gene to a segment of DNA (called the J or joining segment) encoding

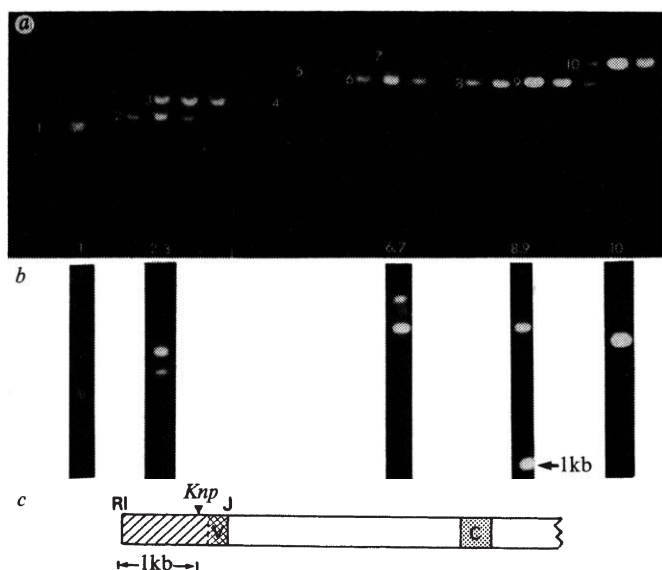


Fig. 1 Identification of the *EcoRI* fragment of embryonic DNA that contains the germ line MOPC-41 variable region gene. *a*, *EcoRI*-digested embryonic DNA was fractionated first by RPC-5 column chromatography and then by agarose gel electrophoresis, transferred to a nitrocellulose filter and hybridised to the ^{32}P -labelled 1 kilobase *EcoRI*-*KpnI* (see map, bottom panel) fragment from the recombined gene as described previously^{1,8}. *b*, Separate fractions from the RPC-5 column containing MOPC-41-like variable-region sequences were pooled as indicated, cleaved with *KpnI*, fractionated on agarose gels, similarly transferred to nitrocellulose filters and hybridised to the ^{32}P -labelled *EcoRI*-*KpnI* fragment. *c*, Location of the sequence within the MOPC-41 recombined gene (the *EcoRI*-*KpnI* fragment) that was used as probe to detect the germ line gene.

these last variable region sequences⁸. Even in the active form, however, the fused V/J segment was separated from the constant region gene by a 3.7 kilobase intervening sequence. Bernard *et al.*⁶ have shown that analogous mechanisms are involved in rearrangement of λ light chain genes, although there may be only one variable region gene for each of the two λ classes.

But many important questions remain. Does the germ line gene undergo substantial somatic mutation before expression? Can recombination between variable and J-segment serve to generate additional diversity? What is the structure of an active, as opposed to an inactive or germ line, immunoglobulin gene? Which sequences define the boundaries of the coding and other functional domains of the gene? And finally, what can these observations tell us about allelic exclusion and/or heavy chain switching?

We have attempted to answer some of these questions by cloning and determining the sequence of major portions of an active κ gene and its germ line precursors. In its germ line form, this sequence is encoded in at least four discrete segments of DNA. A somatic recombination event joins two of these segments, creating the expressed gene which then consists of three discontinuous coding segments. This recombination occurs between one of many germ line variable region genes and one of five short joining regions that we find close to the κ constant region gene¹⁸. The adjoining or J-region is especially interesting in that it must encode two specialised recombination sites, one for DNA joining and another for RNA splicing. Apart from this recombination event, the sequence of the expressed variable region gene is identical to the germ line sequence, that is, no somatic mutation has occurred. A comparison of these sequences with other κ and λ genes suggests that this general structure and mechanism have been preserved in evolution. Further, it suggests that antibody diversity arises principally from three sources: a large and diverse germ line repertoire of

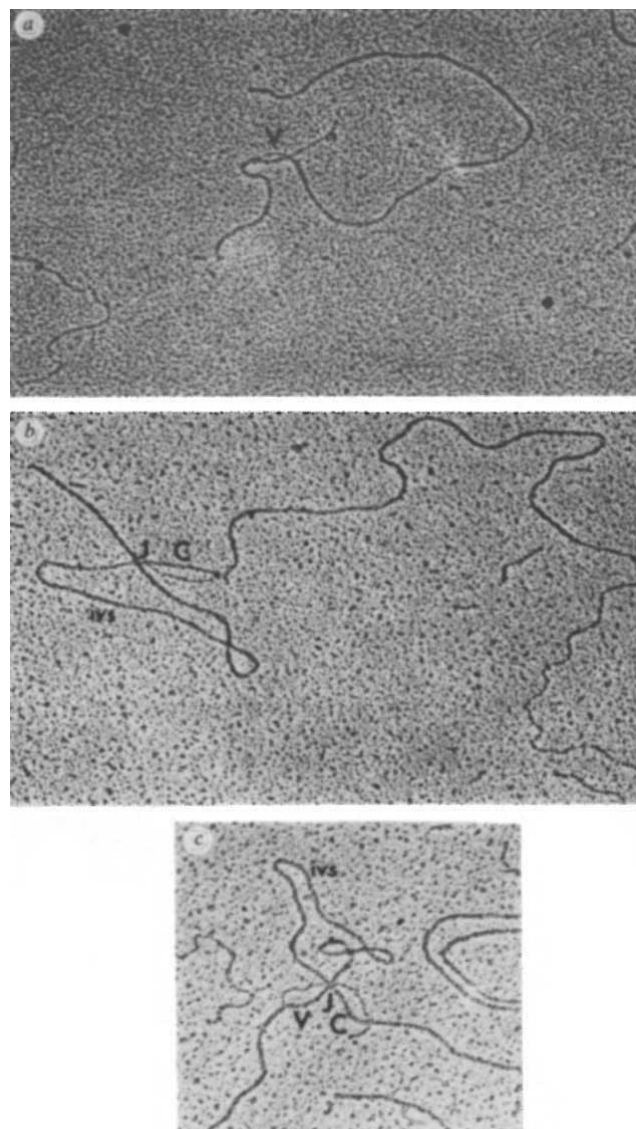


Fig. 2 R-loops formed between MOPC-41 light chain mRNA and the cloned germ line and recombined MOPC-41 light chain genes. Panels represent R-loops formed between MOPC-41 mRNA and the following: *a*, MOPC-41 variable region fragment derived from embryonic DNA (6.8 kilobases); *b*, constant and J region fragment derived from embryonic DNA (16 kilobases); and *c*, fragment derived from MOPC-41 DNA (16.5 kilobases) that contains recombined variable and constant region genes⁸. The R-loops corresponding to the variable region (V), J region (J) and constant region (C) are indicated. IVS identifies the 3.7 kilobase intervening sequence between J region and C region. At least 20 molecules of each type were measured to determine the distances of the genes from one another, and from the ends of the fragments. The R-loops shown in panels *b* and *c* have been published previously⁸ and are shown for comparison. The methods of R-loop formation and characterisation have been described previously⁸. The structures were confirmed by direct sequence determination (see Fig. 4).

variable region genes, a smaller set of J-regions, and a recombination mechanism that joins these sequences in different combinations in the somatic cell.

Cloning the germ line precursor of the active MOPC-41 κ chain gene

The complete MOPC-41 κ chain gene is formed by a recombination event that joins the germ line variable region sequence to a J-region segment located about 3.7 kilobases from the κ constant region gene⁸. We have previously cloned the germ line J and constant region genes on a single segment of embryonic

DNA. We have also cloned the recombinant MOPC-41 gene on a segment of DNA derived from a MOPC-41 plasmacytoma⁸. These clones constitute the product, but only one of the two precursors involved in the formation of the light chain gene. A difficulty arose in cloning the remaining germ line precursor, the variable region gene. It—like other κ variable region genes—is a member of a small set of approximately 10 closely related genes, all of which cross-hybridise to a MOPC-41 variable region probe¹. Fortunately, the parental fragment could be distinguished from the other related sequences by restriction endonuclease digestion (Fig. 1). The recombinant clone had a characteristic *KpnI* site which occurred 1 kilobase from the *EcoRI* site at the 5' end of the variable region sequence. Only one of the ten hybridising *EcoRI* fragments of embryonic DNA (that were separated by two-dimensional analysis¹ and detected by *in situ* hybridisation¹²) yielded a 1 kilobase fragment after appropriate digestion. The fragment (no. 8 in Fig. 1) was tentatively identified as the parental variable region gene.

The partially purified fragment was cloned in λ gtWES- λ B (ref. 13) and analysed by visualisation of R-loop structures formed by annealing MOPC-41 κ chain mRNA to the cloned fragment (Fig. 2). A single variable region R-loop was visualised approximately 1 kilobase from the 5' end of the fragment. R-loop structures formed between the germ line J/constant region fragment and the active, recombinant gene are shown for comparison (Fig. 2). The separate variable and constant region genes, on separate *EcoRI* DNA fragments in the germ line (Fig. 2a and b) are recombined so as to reside on the same DNA fragment in the antibody producing cell (Fig. 2c).

Determination of the nucleotide sequences of the germ line and rearranged genes

Although our electronmicroscopic and restriction enzyme analyses were consistent with a recombination event having occurred between the two parental genes, the fine structure of

the precursor genes and their recombination product as well as the nature of somatic changes, if any, that accompanied the recombination event could only be shown by direct sequence determination. The procedure used in determining the sequence was the partial chemical degradation method of Maxam and Gilbert¹⁴. The restriction enzyme strategy, the overlapping sequences determined and the extent of bi-directional sequencing are shown in Fig. 3. The sequences determined are shown in Fig. 4.

The germ line MOPC-41 variable region gene: evolutionary conservation

Inasmuch as the amino acid sequence of both the mature MOPC-41 protein¹⁵ and its hydrophobic leader¹⁶ have been determined, the coding sequence is easily identified within the cloned fragment. The germ line MOPC-41 variable region gene (VK41) is arranged in a fashion analogous to that of the mouse germ line λ I and λ II variable region genes^{6,9} and other sequenced germ line κ variable region genes (VK2 and VK3)⁴. An 18 amino acid hydrophobic leader coding sequence is separated by a 129 base-pair intervening sequence from the main variable region coding block. [We do not believe that the in-phase initiation codon present close to the analogue of codon -4 in K2 and K3 is the true initiation codon⁴. Rather, further sequencing has revealed that the VK2 gene also has a separately encoded hydrophobic leader sequence (Y. Nishioka, unpublished).] The coding sequence resumes at coding position -4 of the leader sequence and continues to position 95 in the variable region. As we have shown for other germ line κ variable region genes⁴, the last 13 amino acid codons commonly associated with the variable region are missing. Thus, the general structure of these genes seems to have been strongly conserved in the hundred or so million years that κ and λ sequences have undergone separate evolutionary development. It is also worth noting that the sequence homologies, both between other λ and

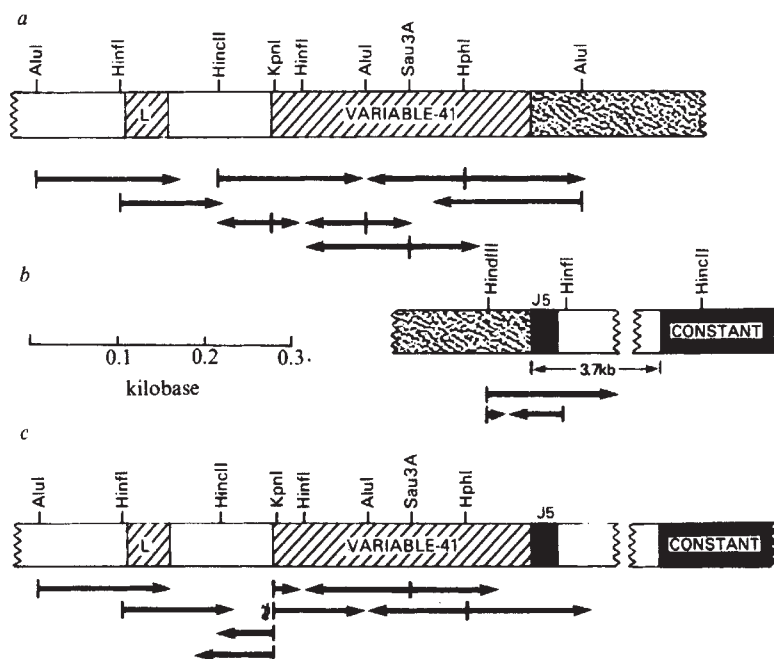


Fig. 3 The strategy used to determine the nucleotide sequences of cloned immunoglobulin genes. The basic approach and methodology have been described by Maxam and Gilbert¹⁴. Our application of their technique has also been described previously⁵. The 6.8 kilobase fragment containing the germ line MOPC-41 variable region gene^{6,8}, the 6.5 kilobase *Bam*HI-*Eco*RI fragment of the 16 kilobase *Eco*RI fragment carrying the germ line J and constant region genes⁸, and the 7 kilobase *Bam*HI-*Eco*RI fragment of the 16.5 kilobase fragment carrying the recombinant gene⁸ were re-cloned in the bacterial plasmid pBR322. Restriction fragments between 500 and 2,400 base pairs were excised from these plasmids by cutting with the enzymes *Eco*RI, *Kpn*I, *Xba*I, *Hind*III and *Bam*HI. (The *Eco*RI site is 1.2 kilobases to the 5' side of the variable region and recombinant genes.) *Xba*I cleaved the embryonic derived fragment 300 base pairs to the 3' side of the variable region gene and 1.2 kilobases to the 3' side of the recombinant gene. *Hind*III cleaved the constant region fragment gene containing fragments between J1 and the constant region gene. *Bam*HI cleaved 1 kilobase to the 3' side of the constant region gene (ref. 8 and our unpublished results). These fragments were cleaved with the indicated enzymes and end-labelled with ³²P by the action of T4 polynucleotide kinase; finally a smaller fragment labelled at only one end was obtained by re-cleaving the labelled fragments with a

third restriction enzyme. The labelled fragments were isolated from polyacrylamide gels and subjected to base-specific chemical degradation and subsequent fractionation on polyacrylamide-urea gels. The derived sequences are indicated in Fig. 4. The accuracy of any sequence is probably greater than 95%; sequences which have been determined in both directions are more accurate than sequences which have been determined in only one direction. The structural gene sequences have been indicated by diagonal lines (leader and variable region) and solid lines (J and constant region genes). The portions of the fragments that are removed by the recombination event are indicated by stippled regions. The clear portion of the fragments are sequences which become the 5' flanking and intervening sequences of the recombinant gene. The scale shows the distance represented by 0.5 kilobase on the cloned fragment.

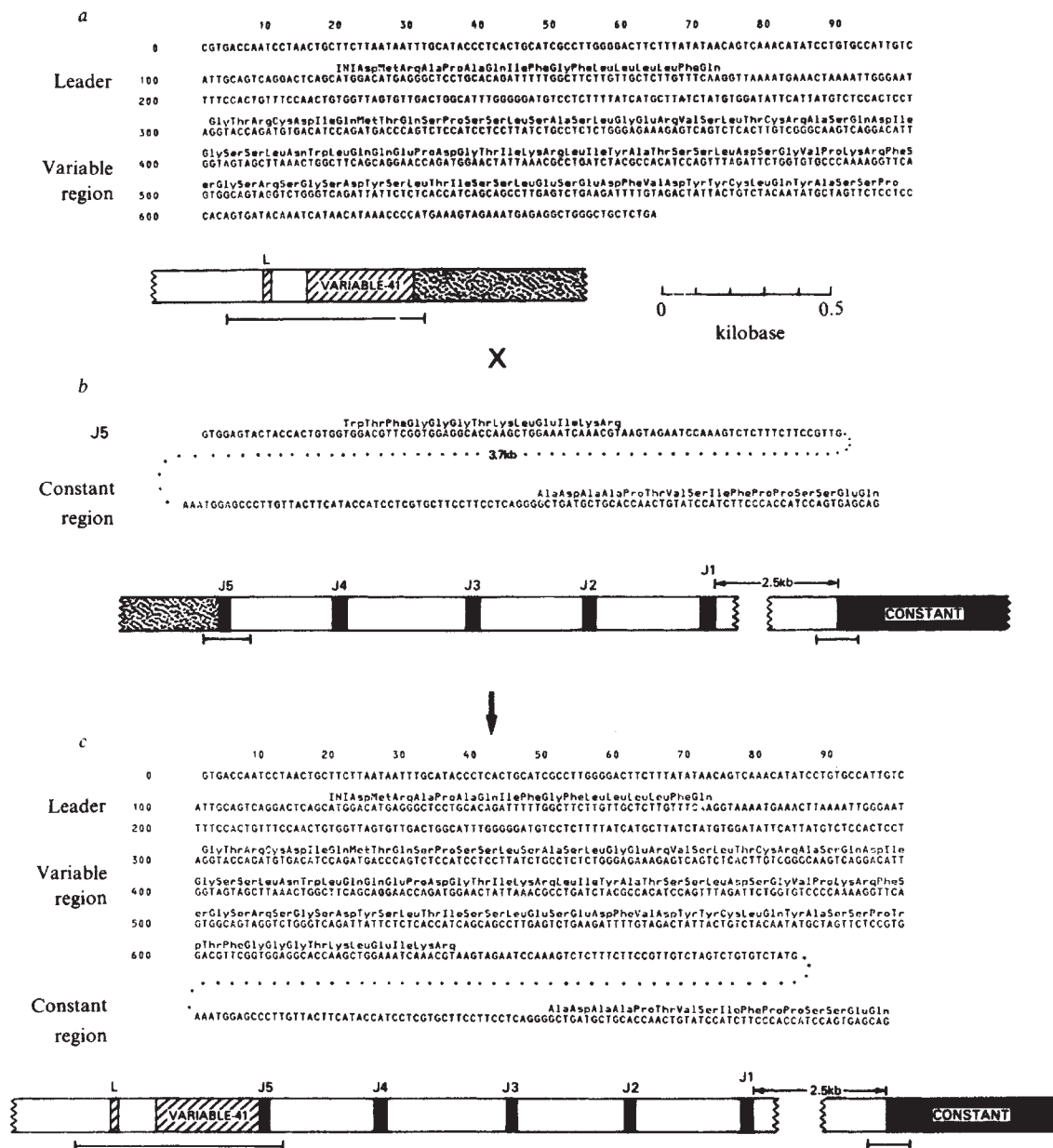


Fig. 4 Nucleotide sequences of the germ line and expressed genes encoding the MOPC-41 light chain. *a*, Germ line leader and variable region gene; *b*, germ line J region and constant region genes (the leader/variable gene and the J/constant gene are encoded on separate *Eco*RI fragments cloned from embryonic DNA); *c*, recombined gene: the light chain gene expressed in the MOPC-41 cell has been formed by fusing the germ line variable and J region genes. Gene segments are diagrammatically coded as described in Fig. 3. The location of each sequence with respect to the structural gene sequence is indicated on the figure below each sequence. The locations of four additional J-region genes, J4, J3, J2 and J1 in the intervening sequence were determined by Max *et al.*¹⁸ using nucleotide sequence analysis.

other κ genes, are most strongly preserved in the coding sequences (85%, VK41/VK2) and least well in the intervening sequences (25%, VK41/VK2), a pattern which was noted originally between closely related β globin genes¹².

The recombination event and the J region: homologies and inverted repeats

As regards the premature termination of the germ line MOPC-41 variable region gene at codon 95, our electronmicroscopic R-loop analysis (Fig. 2) suggests that the remaining codons occurred at a region 3.7 kilobases from the germ line constant region gene. In fact, direct sequence analysis confirmed that the J coding sequence occurs in this region, extending from codon 96 (Trp-TGG) to 108 (Arg-CGT). The precise point at which the recombination event occurred can also be specified by comparing the sequences of the germ line genes to that of the

recombined gene (Fig. 5). The codon in position 95 (Pro-CCT) of the germ line variable region gene has been converted to a different proline codon (CCG) by the recombination event. The G in the third position is therefore derived from the J-region fragment. Obviously a shift of the recombination site would create a different codon and this could be a source of additional immunoglobulin diversity (see below and ref. 18).

The nature of all recombination events between V and J regions cannot be confidently inferred from the single recombination event analysed here. However, our further sequence studies located at least four additional and different potential J sequences between the germ line MOPC-41 J and the constant region gene¹⁸. These sequences occur at regular intervals about 320–360 nucleotides apart and are indicated in the map shown in Fig. 4. Clearly, if all combinations are permitted, considerable diversity can be generated by simply combining the multiple variable region germ line genes with these J segments. This

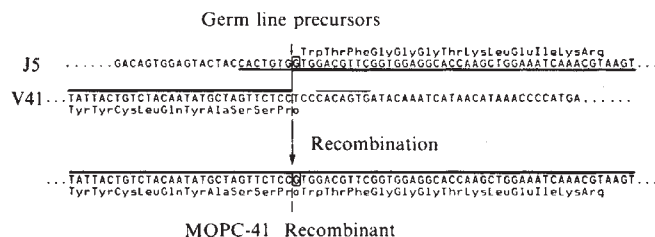


Fig. 5 Nucleotide sequences involved in the recombination of germ line precursors of the active MOPC-41 gene. The precise location of the recombination event is indicated by the cross-over line. The amino acid sequences encoded by each segment are indicated. The locations of a 7 base pair sequence to the 5' side of the J region and a homologous 7 base pair sequence to the 3' side of the variable region are underlined. The sequence is also an inverted repeat; that is, the V region segment is complementary to the J-region segment. Notice that the recombination event occurs in the middle of a proline codon, generating a new proline codon.

possibility has been suggested by a comparison of amino acid sequences of κ light chains in two inbred strains of mice^{19,20}. Diversity would be even more greatly amplified if the recombination event could use different splicing frames at each V/J junction¹⁸.

A comparison of the MOPC-41 variable and J sequences indicates that the recombination event does not depend on simple generalised recombination such as that which requires extensive, in-phase sequence homology. There are several short regions of homology in the variable and J-region, but none align the two sequences in phase. Perhaps one of the most attractive of these homologies is a seven base sequence CAC($\uparrow$)GTG which occurs immediately adjacent to the splice site in both variable and J regions (see underlined sequences, Fig. 5). The sequences are short palindromes or inverted repeat sequences and interestingly appear to border all variable and J regions thus far sequenced¹⁸. Because these are inverted repeats, they are mutually complementary and can be drawn as a short stem structure between V and J genes. We have considered the implication of this possibility and specific molecular mechanisms for recombination in detail elsewhere¹⁸.

It is also likely to be significant that the J-region structure has been closely preserved between κ and λ genes. We have compared our κ J-region sequence to that determined for the λ (type I) J by Bernard *et al.*⁶ (Fig. 6) and find that not only are they identical in length but they are also closely homologous in sequence. In fact, the homologous inverted repeat sequence referred to above is largely preserved adjacent to the recombination site in both the λ J and variable region genes (Fig. 6). It is simplest—perhaps too simple—to think of such sites as recognition core sequences for specialised, site-specific recombination. Nevertheless, these small inverted repeats are formally analogous to the inverted repeats seen at the ends of bacterial insertion sequences^{21,22}.

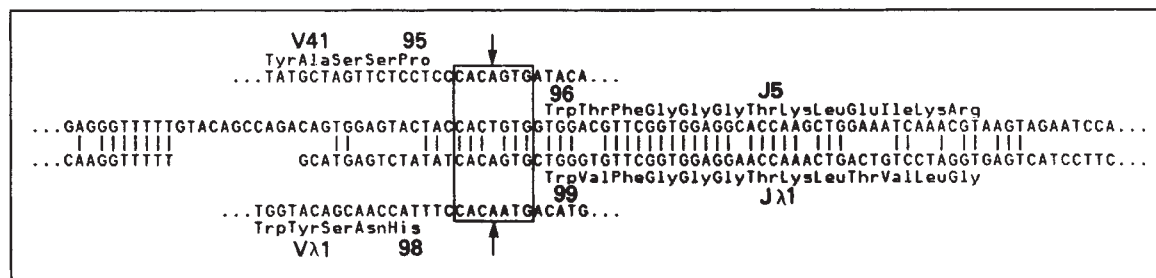


Fig. 6 Nucleotide sequence homologies between κ and λ variable and J-region genes surrounding the DNA recombination site. J1 and J λ 1 have been aligned to indicate the close relationship between these two DNA sequences (homologies are indicated by vertical lines). The 3' ends of the κ (MOPC-41) and λ I variable region genes are indicated so that the location of the common sequence, CAC($\uparrow$)GTG, is aligned (boxed sequence). The arrows indicate the centre of this small palindrome that constitutes an inverted repeat noted in Fig. 5 and present in both κ and λ variable and J-region genes. The λ gene sequences are from Bernard *et al.*⁶.

Except for recombination, the germ line variable region sequence is unaltered in the active gene

Whereas one of a large number of germ line κ variable region genes recombining with one of several J-segments might suffice to generate the complete κ chain repertoire, studies of λ light chains strongly suggest that point mutations arise somatically, producing additional diversity²³. Sequence determination of λ light chain genes⁶ supports the notion that simple somatic base alterations are responsible for the amino acid differences noted among λ chains. Still, even in the λ chain system, a predominant sequence ascribed to the unaltered germ line gene was seen in about two-thirds of the chains examined²³. Although the single position changes noted in myeloma proteins would increase the light chain repertoire and might be relevant to the immune response (they might also be irrelevant, the result of a transcriptionally active gene being repeatedly replicated during prolonged passage in a tumour), the germ line sequence remains the major λ sequence expressed.

Such protein sequence comparisons have been much more difficult in the mouse κ system, for—in contrast to the λ light chains—mouse κ light chains are extremely diverse in sequence, a fact that is consistent with their role as the major (>95%) light chain in all mouse antibodies. This diversity is further consistent with a large number of germ line variable region genes^{1,6,11}. It was therefore of particular interest to determine whether additional somatic mutations had occurred in the germ line VK41 gene. A comparison of the germ line and expressed sequences (Fig. 4) indicates that—with the exception of the V/J recombination event—they are identical in every position. The expressed gene has, therefore, undergone no further somatic alteration.

The recombined MOPC-41 gene remains an interrupted gene: intervening sequences

The large intervening sequence (IVS-2) that separates the recombined variable-J sequence from the constant region gene in MOPC-41 DNA was first identified by visualising R-loop structures formed between the gene and its mRNA (ref. 8). Both this sequence and the smaller intervening sequence (IVS-1) that separates the major portion of the leader from the variable region and are strictly analogous to the corresponding structures of the λ light chain gene^{6,7}. Their borders are established by our nucleotide sequence analysis and these seem to occur within codon -4 and within codon 108 for the small and large intervening sequences, respectively (Fig. 4). Evidence from the mouse globin system²⁵⁻²⁷, the ovalbumin system^{28,29} as well as the immunoglobulin system³⁰⁻³², indicates that these intervening sequences are transcribed and spliced out during maturation of a large mRNA precursor. The nature of the RNA splice signal is not yet known, as there is limited conservation of sequence between these sites among different genes. However, as a general rule, the data are consistent with the elimination of

sequence beginning with [GT] and ending with [AG] (ref. 28). The sequence of the κ light chain gene is consistent with this rule as follows:

IVS-1, CAA G(GT . . . AG)GT ACC,

IVS-2, AAA C(GT . . . AG)GG GCT

where the underlined sequences represent codons and the sequences within parentheses, excised segments.

Antibody diversity, allelic exclusion and the heavy chain switch

We have discussed the role that recombination might play in creating antibody diversity—joining one of hundreds of variable regions to one of several J-segments, each of which is linked, in turn, to the single constant region gene¹⁸. It is clear that this recombination event is critically linked to antibody gene expression. If recombination occurs (or occurs properly) on only one of a pair of alleles, presumably only the recombined gene will be expressed. Allelic exclusion may therefore be a

consequence of a single authentic recombination event affecting only one member of a chromosome pair⁸. Preliminary evidence suggests that germ line genes and/or aberrant recombinants occur together with active genes in myeloma tumours^{8,33}.

The problem of moving a single heavy chain variable region from one heavy chain constant region to another—the so-called heavy chain switch—may also involve the use of a J-like segment and a site-specific recombination event. However, the need to distinguish a stable recombinant (as in a light chain gene) from an unstable one (as in a μ chain gene) suggests that two independent rearrangements are involved in switching from a μ to a γ or an α chain. The first is probably analogous to what we see in the light chain gene, but the second might be quite different, involving an alternative mechanism of DNA rearrangement.

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Persistent emission from X-ray burst sources and the nature of galactic bulge X-ray sources

TWO principal types of theories have been proposed to explain X-ray bursts: instabilities in the accretion flow of matter onto a collapsed object^{1–9}, and thermonuclear flashes in the freshly accreted material on the surface of a neutron star^{10–16}. Both mechanisms probably occur in nature but account for different observed phenomena¹⁷. The available evidence^{17–19} leaves little doubt that the Type II bursts¹⁷, which are emitted by MXB1730–335 (the Rapid Burster), are due to an accretion instability. The properties of Type I bursts¹⁷, which are emitted by all burst sources (including the Rapid Burster), are naturally explained by the thermonuclear flash model¹⁵. In the light of this model, we discuss here the intrinsic persistent X-ray luminosity and the ratio of the average persistent X-ray luminosity to the time-averaged burst luminosity associated with Type I burst sources^{17,20,21}.

Following van Paradijs²², we assume that the peak burst luminosity is a standard candle. The intrinsic persistent X-ray luminosity L_x in units of this standard candle is thus given by the ratio γ of the persistent X-ray flux to the average peak burst flux. On the basis of the results given below we argue that the bright non-bursting galactic bulge X-ray sources^{23,24} are accreting

neutron stars, which differ from X-ray burst sources in that L_x is above a certain critical value.

An earlier compilation of values of the ratio, α , of the average persistent X-ray luminosity to the time-averaged burst luminosity has been given by Lewin²⁰; the α -values presented in Table 1 include more X-ray burst sources and are based on a larger collection of SAS 3 data on all well-studied burst sources. We confirm the earlier conclusion^{15,21} that in general the observed values of α are consistent with the thermonuclear flash model.

Detailed calculations of thermonuclear processes in the surface layers of an accreting neutron star were recently reported by Joss¹⁵ (see also refs 14 and 16). Joss's results indicate that such objects undergo helium-burning flashes, which are visible to an external observer as bursts of X rays. This model can account for most of the basic features of Type I X-ray bursts, and leads to the following predictions concerning the persistent X-ray luminosity of the Type I burst sources: (1) every burst source is also a source of persistent (accretion-driven) X-ray emission; hence, apart from efficiency factors¹², α is just the ratio of the gravitational potential energy liberated by accretion to the nuclear energy released in flashes and should have a value of ~ 100 (refs 12, 13, 15); and (2) bursts occur only when the accretion rate is below a certain critical value and the corresponding persistent luminosity is below a critical value L_c . This effect can be qualitatively understood as resulting from an increase in the pre-flash temperature and a decrease in the level

of degeneracy of the helium-burning shell, and from a decrease of the temperature gradient above the helium-burning shell, with increasing mass accretion rate²⁵. The precise value of L_c is not yet well determined and may depend on other parameters (such as the strength of the neutron-star magnetic field). However, the available calculations^{12,15} suggest that $L_c \approx 0.1 L_{ed}$, where

$$L_{ed} = \frac{4\pi cGM}{\kappa} \approx 1.25 \times 10^{38} \left(\frac{M}{M_\odot} \right) \left(\frac{\kappa}{0.4 \text{ cm}^2 \text{ g}^{-1}} \right)^{-1} \text{ erg s}^{-1} \quad (1)$$

is the Eddington luminosity for an object of mass M and an opacity coefficient of κ (normalised to Thomson scattering opacity in pure hydrogen). Bursts also should not occur when the accretion rate is below another critical value (at least two orders of magnitude smaller than L_c)¹³, because the helium-burning will then be driven by pycnonuclear reactions¹³.

In Table 1 we have collected the values of the observed persistent X-ray flux F_{per} and the ratios α and γ for 10 burst sources. Most of these results are based on data obtained from pointed SAS 3 Y-axis^{26,27} observations; previous data sources are also listed in Table 1.

The data on MXB1820–30 have been taken from the SAS 3 rotation modulation collimator observations by Clark *et al.*²⁸. The upper limit to the persistent X-ray flux from MXB1659–29 has been obtained from changes in the observed X-ray count rate when the source enters and leaves Earth occultation^{18,21}. In the case of the Rapid Burster, we have used the time-averaged Type II burst flux for the persistent flux, as the Type II bursts presumably¹⁷ replace the persistent flux. In April 1977, the observed upper limit to a truly persistent X-ray flux from this source was 20% of the average Type II burst flux²⁹. For the faint X-ray source MXB1906+00, a determination of the persistent X-ray flux from the SAS 3 observations is difficult (due to contamination by Aql X-1 in the field of view²⁶); we therefore used the average persistent X-ray flux of Seward *et al.*³⁰. Because of source confusion problems and the transient character of the possible persistent X-ray counterparts, we have not included in Table 1 the three galactic centre burst sources^{31–34}. Two sources (MXB1659–29 and MXB1820–30) became inactive when F_{per} rose above a certain value^{28,35}; the corresponding values of γ are also given in Table 1. When different values of α were measured during observations of a given source, the range of observed values is presented.

According to calculations by Joss^{12,15}, the maximum luminosity in an X-ray burst is comparable to the Eddington limit. This result is supported by the empirical results obtained by van Paradijs²², which strongly suggest that the average maximum luminosity L_0 of X-ray bursts is indeed about the same for all burst sources and is of the same order as the Eddington limit. Scaling their distances such that the burst sources are distributed symmetrically about the galactic centre yields $L_0 \sim 2 \times 10^{38} \text{ erg cm}^{-2} \text{ s}^{-1}$. The intrinsic persistent X-ray luminosity L_x of a given burst source, in units of the luminosity standard L_0 , is given by γ (see Table 1).

The following trends are visible in the distribution of the values of γ . First, the sources all have $\gamma < 0.3$ when active. This is consistent with the values for which the calculations by Joss¹⁵ and Joss and Li¹⁶ predict bursting behaviour. Second, the two sources which show the most irregular burst behaviour (MXB1735–44 and MXB1837+05^{18,36–38}) have the largest values of γ of all sources in Table 1. The irregular burst behaviour of MXB1735–44 and MXB1837+05 may be related to the rather large persistent luminosity of these sources, which may lie near the critical value above which X-ray bursts are expected to be absent. (Note, however, that the behaviour of X-ray burst sources must depend on additional complications beyond these general considerations; in particular, there are few correlations between the level of burst activity and the instantaneous persistent X-ray luminosity in burst sources^{37–40}).

The values of α given in Table 1 are averages over time intervals of typically a few days. For most of the sources α is a relatively well defined quantity; the average total energy per

Table 1 X-ray burst source parameters

Source	F_{per}^* ($\text{erg cm}^{-2} \text{ s}^{-1}$)	α	γ	Refs F. Li (personal communication)
MXB 0512–40	8.0 (–10)	75	0.05	
MXB 1636–53	6.3 (–9)	300; 50‡	0.09	22, 40
MXB 1659–29	<3.9 (–10); 1.7 (–9)†	<25	<0.04; 0.15‡	17, 21, 22
MXB 1728–34	3.9 (–9)	120	0.05	20, 22, 39
MXB 1730–335	1.2 (–9)§	130	0.06	18, 19, 21
MXB 1735–44	5.1 (–9)	380–1580; 85–200‡	0.18	22, 36, 37
MXB 1820–30	1.5–4.0 (–9); 6.0 (–9)†	40–100	0.01–0.15; 0.06‡	20, 28
MXB 1837+05	9.1 (–9)	780–1100; 190‡	0.30	20, 22, 38
MXB 1906+00	2.1 (–10)	70	0.08	20, 22
MXB 1916–05	2.8 (–9)	270	0.13	22, 49

* Numbers in parentheses indicate powers of 10.

† No bursts observed above this value of the persistent X-ray flux.

‡ When two values are shown, the latter corresponds to intervals of high burst activity.

§ Time-averaged Type II burst flux.

burst from a given source, the time intervals between them and the value of the persistent flux do not vary greatly. We estimate that the values of α typically have a possible uncertainty of $\approx 50\%$. In some cases, however, the burst behaviour is quite irregular. An example is the source MXB1735–44, where the burst intervals vary from ~ 1 –2 h to more than 50 h^{18,21,36,37}. In those cases where appreciable irregularity of the burst activity is apparent, we have given separately in Table 1 the values of α during times of exceptional burst activity.

Very high values of α are obtained for the irregular sources MXB1735–44 and MXB1837+05, when averaged over the entire period of observation; values between 100 and 200 are observed, however, during the periods of high burst activity. In the context of the thermonuclear flash model, these values for α suggest that during periods of burst inactivity the material in the freshly accreted layers on the neutron star undergoes relatively steady nuclear burning. This result is strengthened by the observation that the energy of the first burst after a long period of burst inactivity is about the same as for the bursts that occur throughout intervals of high burst activity. It may not, therefore, be necessary to invoke the “storage model,” introduced by Woosley and Taam¹⁰, in which nuclear fuel is accumulated during burst-inactive periods and is consumed only during periods of burst activity.

With one exception, the values of α in Table 1 fall in the range of ~ 50 – ~ 200 (during periods of high burst activity) which agrees well with the values predicted by the thermonuclear flash model. The low upper limit of 25 in the case of MXB1659–29 may perhaps result from bolometric corrections and anisotropic emission of the persistent flux^{12,41,42}, or from the mixing of hydrogen into the helium-burning shell¹⁴.

Lamb and Lamb¹³ suggested that the observed values of α were too small to be consistent with any thermonuclear flash mechanism, unless one invokes the “storage model” described above. Our conclusions differ from those of Lamb and Lamb¹³ principally for the following reasons: (1) More detailed computations¹⁵ indicate that the nuclear processing in a helium-burning flash proceeds all the way to iron-peak nuclei, thereby releasing ~ 2.7 times more energy per unit mass than in the simple fusion of helium into carbon. (2) We exclude the Type II bursts from the Rapid Burster, as these bursts are produced by a different physical mechanism than that responsible for Type I bursts¹⁷. (3) Preliminary estimates (see Lamb and Lamb¹³) of the

values of α from some sources were lower than the more accurate values given in Table 1.

The burst sources resemble the bright galactic bulge X-ray sources^{23,24} in their galactic distribution⁴³ and in the spectra of their persistent X-ray fluxes^{44,45}. In fact, some of the steady counterparts of the burst sources (such as MXB1735–44) can be categorised as bulge sources (see refs 24, 46).

Lewin *et al.*⁴³ suggested that the galactic distribution of X-ray burst sources was consistent with their being a sub-class of already known low galactic latitude X-ray sources, while Ostriker⁴⁴ and Markert *et al.*⁴⁵ have proposed that the burst sources are a subset of the galactic bulge and globular cluster X-ray sources on the basis of their spectral properties. However, the brightest of the galactic bulge sources, with luminosities of $\sim 10^{38}$ erg s⁻¹, have never been found to burst.

The classification of the galactic bulge sources into two groups by Parsignault and Grindlay⁴⁷, which is based on their X-ray spectra (Fe line emission) and the character of their brightness variations, largely coincides with a grouping of these sources according to absolute X-ray luminosity. This is shown by histograms of the apparent brightness⁴⁸ of the sources in these two classes, given that the galactic longitude distribution of the sources ($|l^{\text{II}}| \leq 20^\circ$) indicates a small range of distances. Apparently the mass accretion rate is one of the most important parameters that determine the variability (including the presence of Type I X-ray bursts) and spectral properties of bulge sources.

In view of the results presented here we suggest more specifically (compare refs 43–45) that the majority of the galactic bulge X-ray sources are neutron stars that are undergoing accretion, and that the burst sources are a subset of the bulge sources whose accretion rate is below a critical value (and, perhaps, with other parameter values that permit bursting behaviour, such as weak magnetic fields¹⁶). For accretion rates above this value, the structure of the freshly accreted envelope on the neutron star is changed such that nuclear burning proceeds relatively steadily. Below this value, thermonuclear flashes occur which are observed as X-ray bursts.

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The ring systems of Jupiter, Saturn and Uranus

THE recent direct observation by the Voyager spacecraft of a ring around Jupiter¹ makes a comparison between the jovian ring and the rings of Saturn and Uranus possible, and leads to certain conclusions about their origins which are reported here.

Studies made by Vsekhsviaty² and described by Struve³ of the position of an irregular dark line on Jupiter with respect to the subsolar point suggested the presence of a ring less than two jovian radii above the surface. This radius falls, however, beyond the Roche limit and beyond the satellite Amalthea. On the other hand Acuna and Ness⁴ proposed that certain minima in the charged particle fluxes observed by Pioneer 11 are caused by a ring at 1.83 times the jovian radius⁵, a value which agrees well with the Voyager results. Comparing the size of the minima caused by the ring with those caused by the nearby Amalthea one can conclude that the solid surface area of the ring should be about 20,000 km² assuming that only the geometrical cross-section plays a part.

It is interesting to compare the location and width of the rings of Jupiter, Saturn and Uranus with the various theoretical limits. Besides the classical Roche⁶ limit a_R , which is defined as the smallest distance at which a liquid self-gravitating body remains spheroidal and does not breakup, there are the limits a_i and a_0 indicating the distances at which an incident or orbiting, spherical, homogeneous and defectless solid body will break up in a most fracture-resistant mode⁷; non-spherical bodies and those containing unavoidable defects will breakup, depending on their mode of fracture, at much larger distances. There are also two accretion limits⁸, the lower one, a_i , denoting the distance below which only molecular accretion onto an orbiting particle is possible and the upper one, a_u , which is the smallest distance at which two particles of the same radii can accrete. For rotating particles, a between a_i and a_u , there is an upper limit of the radius of the larger particle onto which a smaller one can accrete. Beyond a_u all modes of accretion are possible and thus larger bodies can form. The various limits can be expressed as $a = C(M/\rho)^{1/3}$ with $C_R = 2.21$, $C_0 = 1.25$, $C_i = 1.08$, $C_1 = 1.31$ and $C_u = 2.62$; here M is the mass of the planet and ρ is the density of the ring particles. Figure 1 shows the location of the rings and the various limits for the three planets are indicated assuming that the rings are made either of ice ($\rho = 1$ g cm⁻³) or of carbonaceous chondrites ($\rho = 3$ g cm⁻³); the location of the nearest satellites is also shown. All the rings lie outside the a_i , a_0 , a_1 , limits some of which are even smaller than the indicated planetary radii. The jovian ring is below the Roche limit for both ice and chondrite, Saturn would agree with this limit if the density of ice and chondrite were 0.77 g cm⁻³ while Uranus would agree if the density were less than 2.2 g cm⁻³. The upper accretion limit a_u is satisfied for both ice and chondrite for Jupiter and Uranus but only for ice for Saturn.

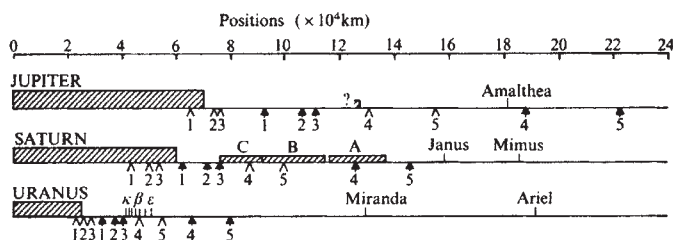


Fig. 1 The positions of the rings of Jupiter, Saturn and Uranus as well as those of their nearest satellites (above the lines) and of the various theoretical limits for densities 1 (▲) and 3 (△) g cm^{-3} (below the lines). The inner boundary of the jovian ring is uncertain. 1, Breakup of incident body a_i ; 2, breakup of orbiting body a_0 ; 3, lower accretion limit a_l ; 4, Roche limit a_R ; 5, upper accretion limit a_u .

A ring which was formed by accretion of particles formed in the protoplanetary nebula would be expected to be located between a_l and a_u to make the process possible and to ensure that continuous collisional comminution does not lead to its disappearance through the action of radiation pressure, the Poynting–Robertson effect, and so on⁹. One would not expect the ring to be outside a_u because in that position accretion should be rapid and lead to the breakup of the ring into larger clumps and bodies. On the other hand a ring which was formed by the breakup of a body under the action of tidal forces should lie near a_l and a_0 only if the body was spherical and broke in a particular fracture-resisting manner⁷. Unless the body were very large, of the order of a few hundred km, one would not expect it to be spherical and it could thus breakup at any distance larger than a_l or a_0 . The resulting ring would be rather narrow, it would tend to be lumpy, uneven in thickness, and if the initial fracture had produced a few larger pieces they may have formed distinct separate rings.

The above considerations agree with the conclusion that the saturnian rings, which fit so well between a_l and a_u , are made primarily of ice and that they are the product of condensation from the protoplanetary nebula and subsequent collisional spread. The 10 or so rings of Uranus are probably the result of a breakup of one or more bodies which were non-spherical and mechanically weak and probably of carbonaceous composition⁹. The formation and maintenance of distinct, separated rings was probably the result of resonances with various satellites¹⁰. The breakup of the body occurred probably not less than 10^7 or 10^8 yr ago because the rings seem to be continuous. The jovian ring is probably an example of a much more recently fragmented body which was non-spherical, mechanically weak and probably also of the carbonaceous kind. As pointed out by Pollack¹¹ the thermal history of Jupiter is unfavourable for the ring to form by condensation. If the width of the ring were 20–200 km, combining it with the total solid surface of the ring of 20,000 km^2 leads to an opacity one or two orders of magnitude lower than that of the weakest rings of Saturn which would explain the observational difficulties.

The tenuous nature of the ring may be due to continuous bombardment and erosion by the intense flux of protons in the jovian Van Allen belt. An estimate of this erosion can be made using a flux $\Phi = 2 \times 10^6 \text{ cm}^{-2} \text{ s}^{-1}$ at 1–2 MeV and assuming $d\Phi/dE \sim E^{-1.5}$ dependence (ref. 12 and J. A. van Allen, personal communication), using Draine and Salpeter's¹³ sputtering yields for MgSiO_3 and graphite and the sputtering yields for ice of Brown *et al.*¹⁴. One obtains an average erosion of 50–100 Å for ice, 2–3 for silicate and 0.2–0.5 Å yr^{-1} for graphite. Many small grains would thus be eliminated from the ring on the time scale of 10^6 yr once their size became small enough to be affected by radiation pressure, the Poynting–Robertson effect, and so on. In these circumstances the ring may show more rapid evolutionary changes than those expected to

occur on the saturnian ring A¹⁵ and may have a much shorter life than the rings of the two other planets.

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Cyclic reaction mechanism in the electrodeposition of aluminium

DENDRITIC growth is one of the most serious problems encountered in the galvanic deposition of metals. For many applications a high deposition rate is required but the rate cannot exceed the transport rate of metal ions to the electrode. A method of solving the transport problem by a cyclic mechanism is presented here, which combines the electrode process with a subsequent chemical reaction.

Metallic layers deposited in diffusion-controlled conditions are incoherent and powdery and are therefore not suitable for normal applications. But even at lower deposition rates the formation of the metallic layer will be influenced by diffusion. Because of the concentration gradient due to the electrode reaction, metallic nuclei that protrude in the depleting solution will grow faster than the others. The roughness of the electrode surface will be magnified and after a certain time dendritic growth will be observed. Levelling agents are added to industrial galvanic solutions to avoid dendrites. In most cases the levelling effect of these agents is due to their adsorption on a growing nucleus and the resulting deactivation of the nucleus. New nuclei have to be formed continuously and the result is a smoothly growing surface.

Instead of poisoning a growing nucleus we propose to solve the transport problem itself by combining the electrode process

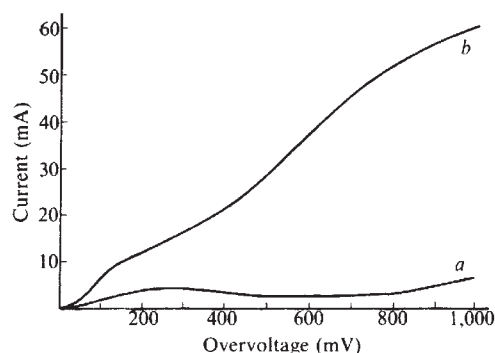


Fig. 1 Current–overvoltages curves for the deposition of aluminium at 25 °C from: a, 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$ in $\text{LiAlH}_4\text{–THF}$; b, 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$, 0.44 M $\text{AlCl}_3 \cdot 2\text{THF}$ in $\text{LiAlH}_4\text{–THF}$.

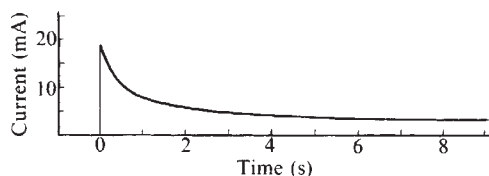


Fig. 2 Chronoamperogram for the deposition of aluminium in a 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$ -LiAlH₄-THF solution at -403 mV and 25 °C.

with a subsequent chemical reaction so that the concentration of the active compound remains constant at the electrode surface:



where A represents the active compound, M the deposited metal and Z an electrochemically inactive compound present in the solution at high concentration and is capable of producing A. In a cyclic process the compound A is reduced at the electrode (1) but regenerated in a fast chemical reaction (2).

Reaction mechanisms of this type are well known in catalysis where a low activation energy is required for the reaction (1)

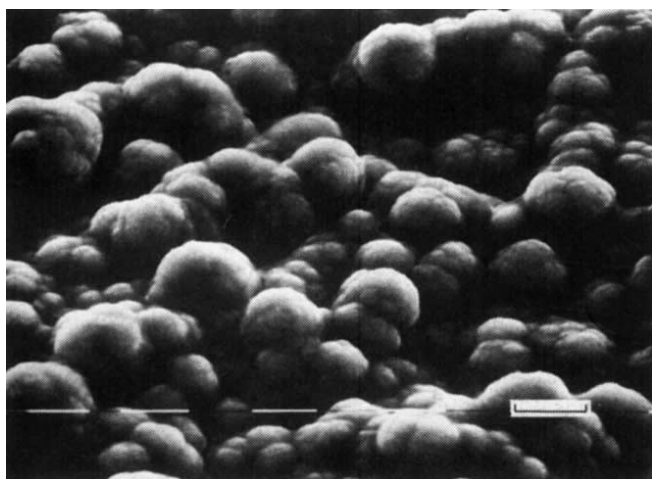
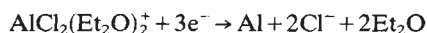


Fig. 3 Scanning electron micrograph of aluminium deposited at 3.5 mA cm⁻² and 25 °C for 90 min from a solution of 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$ in LiAlH₄-THF. Scale bar, 10 μm.

promoting the conversion of Z into M. To obtain a cyclic electrodeposition process it is essential that the overpotential for the deposition of M from A (1) is smaller than that for the reduction of Z into M.

For the electrodeposition of aluminium Brenner¹ introduced a solution of LiAlH₄ and AlCl₃ in diethylether (Et₂O). Very pure aluminium with a high cathodic efficiency can be obtained with this solution. Levinskas² proposed the following reaction for the electrode process:



We think, however, that the electrode process occurs by means of a cyclic mechanism as given in reactions (1 + 2) where AlH_3 is the active compound 'A' in the electrodeposition. The compound is regenerated in the reaction of $\text{AlCl}_2 \cdot (\text{Et}_2\text{O})_2^+$ (Z) with hydride ions (B). The first evidence is the observation that no aluminium metal can be deposited from a solution of AlCl₃ in diethylether without decomposing the solvent. This rules out the

scheme of Levinskas. In contrast very pure aluminium is deposited from a solution of $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$, LiAlH₄ in diethylether³. This shows that the overpotential for the aluminium deposition is larger for AlCl₃ than for AlH_3 . The deposition rate is limited by mass transport as shown from the diffusion plateau of the current-overvoltage curve (Fig. 1a). The chronoamperogram (Fig. 2) measured at -403 mV also shows this diffusion dependence.

The variation of the current as a function of time is in good agreement with the Cottrell equation:

$$i = \frac{nFSD_A^{1/2}C_A^0}{(\pi t)^{1/2}}$$

Electrodeposition at a current density of 3.5 mA cm⁻² results in a rough and spongy surface as shown in Fig. 3. This structure is a precursor of dendritic growth.

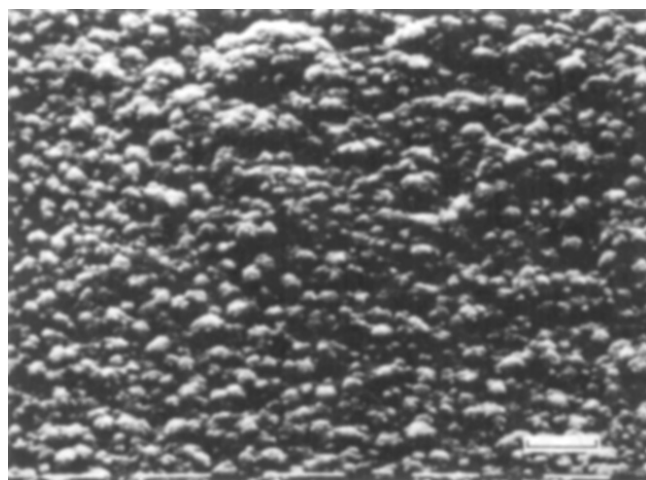


Fig. 4 Scanning electron micrograph of aluminium deposited at 21 mA cm⁻² and 25 °C for 15 min from a solution of 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$, 0.44 M AlCl₃ · 2THF in LiAlH₄-THF. Scale bar, 10 μm.

The compound AlH_3 was obtained in a pure and crystalline form as an adduct with triethylamine: $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$. For the electrochemical measurements the less volatile tetrahydrofuran (THF) was used instead of diethylether as the solvent. The measurements were carried out with a special cell design in which the aluminium reference electrode was located in a cavity behind the working electrode ensuring a very small ohmic drop between working and reference electrodes.

When AlCl₃ · 2THF is added to the previous solution of $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$, LiAlH₄ in THF the situation is completely changed. There is no diffusion plateau in the voltammogram

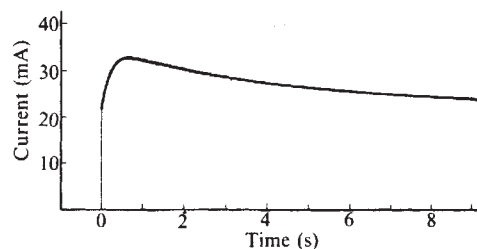
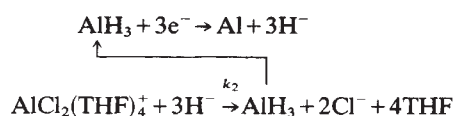


Fig. 5 Chronoamperogram for the deposition of aluminium in a 0.17 M $\text{AlH}_3 \cdot 2\text{Et}_3\text{N}$, 0.44 M AlCl₃ · 2THF solution at -403 mV and 25 °C.

(Fig. 1b) and compact aluminium layers are obtained even at higher current densities (Fig. 4).

The chronoamperogram (Fig. 5), also measured at -403 mV, indicates that in this case the current is no longer diffusion controlled. We explain this by the following cyclic process:



The chemical reaction maintains a constant concentration of the active compound at the electrode and the result of the process is the deposition of a compact aluminium layer. The measured values of the current as a function of time are in good agreement with the equations derived by Delahay⁴.

$$i = nFSD_A^{1/2} C_A^0 (k_2 C_Z^0)^{1/2} \text{erf} (k_2 C_Z^0 t)^{1/2} + \frac{nFSD_A^{1/2} C_A^0}{(\pi t)^{1/2}} \exp(-k_2 C_Z^0 t)$$

The rate constant $k_2 = 11.7 \text{ s}^{-1} \text{ mol}^{-1}$ (in THF at 25°C) of the chemical process can be calculated from the chronoamperometric measurements. The value of the rate constant k_2 , together with the concentration of AlCl_3 (C_Z^0), defines the limits between which the cyclic process can function.

We believe that a cyclic mechanism can also explain the effect of adding cyanide compounds to an aqueous solution used for the electro-deposition of zinc. It is found⁵ that $\text{Zn}(\text{OH})_2$ is the active compound in the electrode reaction but it is also known that the presence of cyanide ions gives smoother deposits than those obtained from zincate alone⁶.

In a cyclic process the diffusion limitation of $\text{Zn}(\text{OH})_2$, which is only present at a low concentration level, is removed by the chemical regeneration of $\text{Zn}(\text{OH})_2$ in the reaction of $\text{Zn}(\text{CN})_4^{2-}$ with hydroxide ions.

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Effects of acetylene and soil water content on emission of nitrous oxide from soils

THERE are fears that increased nitrogen fertilisation of soils may increase release of N_2O from soils through denitrification of nitrate and thereby pose a threat to the stratospheric ozone layer protecting the Earth from biologically harmful UV radiation from the Sun^{1–6}.

Most of the fertiliser nitrogen added to soils is in the form of ammonium or of ammonium-producing compounds such as urea. Before this nitrogen can be converted to N_2O by denitrifying microorganisms, it must be oxidised to nitrite or nitrate by the nitrifying microorganisms in soils. Studies with pure cultures⁷ have shown that at least one of these nitrifying microorganisms (*Nitrosomonas europaea*) can produce N_2O during oxidation of ammonium to nitrite, so the possibility that nitrifying as well as denitrifying microorganisms contribute to N_2O emissions from soils must be considered. Recent work^{8,9} has shown that N_2O emissions from well aerated soils treated with

ammonium sulphate greatly exceeded N_2O emissions from the same soils treated with an equivalent amount of nitrogen as potassium nitrate and were greatly reduced by addition of nitrapyrin [2-chloro-6-(trichloromethyl)-pyridine], which inhibits nitrification of ammonium in soils^{10,11} but has little or no effect on denitrification of nitrate by soil microorganisms¹². These observations indicated that most of the N_2O evolved from well aerated soils treated with ammonium or ammonium-producing fertilisers is formed during oxidation of ammonium to nitrite by nitrifying soil microorganisms and that these microorganisms contribute significantly to N_2O emissions from soils. We report here further evidence for these conclusions. This evidence was obtained by studying the effects of acetylene (C_2H_2) on N_2O emissions from soils incubated at different water tensions after treatment with ammonium sulphate or potassium nitrate. The effects of C_2H_2 were studied because recent work has shown that this gas inhibits reduction of N_2O to N_2 by denitrifying microorganisms in soils or pure cultures without affecting reduction of nitrate to N_2O by these microorganisms^{13–17} and also inhibits oxidation of ammonium by *Nitrosomonas europaea*¹⁸ and the nitrifying microorganisms in soils¹⁹.

The soils used (Table 1) were screened (<2 mm) samples of field-moist surface soils selected to obtain a range in properties. Subsamples containing 30 g of soil were incubated at 30°C after treatment with ammonium sulphate or potassium nitrate ($100 \mu\text{g N per g}$) and adjustment of their water contents to the equivalent of 0, 10 or 100 cm of water tension. Incubations were performed under air or air containing 0.1% (v/v) C_2H_2 in 1.2-l glass bottles fitted with ground glass joints and glass stopcocks, and the atmospheres in the bottles were sampled for N_2O analysis and renewed after various times. Nitrous oxide was determined by a gas chromatographic technique that utilises the xenon in air as an internal standard²⁰. Soil water tension was determined by the tensiometer described by Rose²¹, and nitrogen as (nitrite + nitrate) present in soils before and after incubation was determined as described by Bremner and Keeney²².

Table 1 shows the effects of C_2H_2 on the amounts of nitrogen as N_2O and as (nitrite + nitrate) produced on incubation of well aerated samples of ammonium-treated soils. Besides confirming that C_2H_2 is a potent inhibitor of nitrification in soils, the data reported show that this gas inhibited production of N_2O in ammonium-treated soils. This is evidence that most, if not all, of the N_2O evolved from these soils in the absence of C_2H_2 was produced by nitrifying microorganisms.

Further evidence for this conclusion is reported in Table 2, which shows the effects of C_2H_2 and soil water content on the amounts of N_2O evolved from soils treated with ammonium sulphate or potassium nitrate. The data reported show that the amounts of N_2O evolved from ammonium-treated soils at water tensions ranging from 0 to 100 cm increased with increase in soil water content and greatly exceeded the amounts evolved from the corresponding nitrate-treated soils. Table 2 also shows that

Table 1 Effects of acetylene on amounts of nitrogen as N_2O and as (nitrite + nitrate) produced on incubation of ammonium-treated soils under aerobic conditions

Soil	pH	Organic carbon (%)	Clay (%)	Sand (%)	N_2O nitrogen evolved in 12 days (ng per g soil)		(Nitrite + nitrate) nitrogen produced in 12 days (μg per g soil)	
					– C_2H_2	+ C_2H_2	– C_2H_2^*	+ C_2H_2^*
Storden	8.2	0.4	12	53	170	<1	83 (4)	<1 (<1)
Harps	8.1	4.2	37	18	208	<1	105 (11)	<1 (<1)
Webster	6.8	3.8	25	28	30	<1	82 (9)	<1 (<1)
Nicollet	6.2	2.6	27	37	24	<1	67 (6)	<1 (<1)

Soils treated with ammonium sulphate ($100 \mu\text{g N per g soil}$) were incubated at 100 cm water tension under air or air containing 0.1% (v/v) C_2H_2 . Atmospheres in incubation flasks were renewed at 3-day intervals.

* Figures in parentheses indicate the amount of nitrogen as (nitrite + nitrate) produced in control (no ammonium added) (μg N per g soil).

Table 2 Effects of soil water tension and acetylene of N₂O emissions from soils treated with ammonium sulphate or potassium nitrate

Soil	Form of N added	Soil water tension (cm water)	N ₂ O nitrogen evolved (ng per g soil)					
			2 days		7 days		14 days	
			-C ₂ H ₂ + C ₂ H ₂	-C ₂ H ₂ + C ₂ H ₂	-C ₂ H ₂ + C ₂ H ₂	-C ₂ H ₂ + C ₂ H ₂	-C ₂ H ₂ + C ₂ H ₂	-C ₂ H ₂ + C ₂ H ₂
Harps	Ammonium	0	625	82	1,170	96	1,175	103
		10	270	2	399	8	401	12
		100	130	<1	202	<1	210	<1
	Nitrate	0	1	71	6	73	7	74
		10	1	1	1	4	2	5
		100	<1	<1	<1	<1	1	<1
Webster	Ammonium	0	34	1	42	2	52	4
		10	29	1	35	1	44	2
		100	18	<1	23	<1	31	<1
	Nitrate	0	2	<1	6	2	6	3
		10	1	<1	3	1	4	2
		100	1	<1	2	<1	3	<1

Soils supplemented with N (100 µg per g soil) as ammonium sulphate or potassium nitrate were incubated at various water tensions under air or air containing 0.1% (v/v) C₂H₂. Atmospheres in incubation flasks were renewed after 2, 4, 7, 9 and 11 days.

C₂H₂ greatly reduced evolution of N₂O from ammonium-treated soils and that the amounts of N₂O evolved from these soils in the presence of C₂H₂ were similar to, or greater than, the amounts evolved from the corresponding nitrate-treated soils. Since C₂H₂ inhibits production of N₂O via oxidation of ammonium by nitrifying microorganisms but promotes release of N₂O during reduction of nitrate by denitrifying microorganisms, these observations seem to leave no doubt that most of the N₂O evolved from the ammonium-treated soils in the work reported was produced during oxidation of ammonium by nitrifying soil microorganisms and that very little of this gas was produced through reduction of nitrate by denitrifying microorganisms.

Our finding that N₂O emissions from ammonium-treated soils increased markedly as the soil water tension decreased (that is, as the soil water content increased and the availability of oxygen to soil microorganisms decreased) is readily explained if nitrifying microorganisms are responsible for these emissions. Ritchie and Nicholas²³ have shown that *Nitrosomonas europaea* produces an enzyme that allows it to reduce nitrite to N₂O during oxidation of ammonium and thereby utilise nitrite as a terminal electron acceptor instead of oxygen when the supply of oxygen is limited.

Our conclusion that nitrifying microorganisms contribute substantially to N₂O emissions from soils is supported by field studies showing that N₂O emissions from plots fertilised with nitrogen as ammonium sulphate exceeded those from corresponding plots treated with an equivalent amount of nitrogen as calcium nitrate (A. D. Matthias, A.M.B. and J.M.B., unpublished).

Other evidence that nitrification deserves much greater attention in research on sources of atmospheric N₂O has been provided by recent work^{24,25} indicating that nitrification may be the major source of N₂O in the oceans.

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Non-uniform vertical distribution of fine sediment in the Amazon River

IN studies of sediment transport in rivers, it is frequently assumed that suspended particles finer than a certain size (usually of the order of 60 µm) are uniformly concentrated from river bed to water surface^{1,2}. The assumption seems to be mostly a matter of convenience; it is well established that the vertical concentration gradient for sediment particles suspended in rivers is directly proportional to fall velocity of the particles and inversely proportional to shear velocity of the flow³. For very fine particles suspended in highly turbulent flows, concentration gradient may be small; the assumption has been used, therefore, to justify collection of near-surface samples in streams where velocities are high and turbulence is intense². Our recent studies of sediment in suspension in the Amazon River show that this assumption is not valid for deep rivers and that appreciable errors result from using concentrations of surface samples to represent concentration of suspended sediment and its associated adsorbed constituents through the entire stream depth.

During high-water seasons of 1976 and 1977, we collected samples of water and sediment in the Amazon River between the mouth and Iquitos, about 3,700 km upriver from the mouth. We used a variety of sampling equipment aboard RV Alpha Helix: Niskin bottles, a point sampler, a depth-integrating

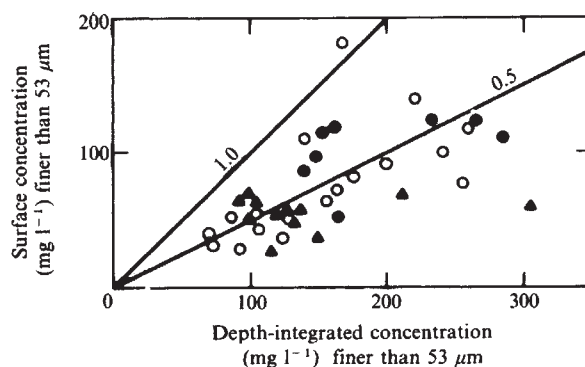


Fig. 1 Relations between concentrations of fine-grained suspended sediment at the river surface and in depth-integrated samples in the Amazon River mainstem near Iquitos, at São Paulo de Olivença, at Santo Antônio do Itá, at Itapeúba (near Coari), near Manacapuru, and at Óbidos. Samples collected between 20 May and 2 June 1977. Points are coded by river depth: ●, depths ≤24 m; ○, 24-49 m; ▲, ≥50 m. Lines through the graph are not regression lines, but are reference lines to show ratios of surface to depth-integrated concentrations.

sampler, and a bucket. The Niskin bottle, which has become standard sampling equipment in most US-based research on the chemistry of the oceans, is lowered open to the desired depth and then closed by a messenger sent down the wire. The point sampler we used is a modified version of the US P-63 sampler that is used in deep rivers of North America²: it has a nozzle that faces directly into the flow and that is designed to admit water at its ambient velocity in the river. The nozzle has a valve that can be opened and closed by remote control after the sampler has been lowered to the desired depth. As the sampler is lowered into the flow, air is compressed in the sample container so the pressure inside the container and in the flow are equal. This is to avoid an initial surge into the sampler when the valve is suddenly opened, which will bias the sample². Our sampler had an auxiliary air chamber to allow pressure equalisation to depths of 100 m. The depth-integrating sampler we used is an enlarged modification of the type of sampler with which most river-sediment samples are collected in the US². It has the same type of nozzle as the point sampler (but without a valve that closes it), and it is lowered to the river bed and back to the surface at a uniform vertical rate. The resulting depth-integrated sample is thereby weighted for differences in velocity in the vertical, and the concentration of sediment in the sample is a velocity-weighted average concentration. Our simplest piece of equipment was a bucket with which we dipped some of the samples from the river surface. Details of our sampling and analytical procedure including a list of our data are given elsewhere^{4,5}.

The particle-size analyses represented in Figs 1 and 2 were carried out by several procedures. Samples collected in 1976 were separated entirely aboard ship by sieve (sizes $>63\ \mu\text{m}$) and pipette (sizes $<63\ \mu\text{m}$). The sieved and pipetted fractions were preserved (latter on preweighed Nucleopore filters having a pore diameter of $0.4\ \mu\text{m}$) for later weighing in the laboratory in Edinburgh. Samples collected in 1977 were all passed through a $53\text{-}\mu\text{m}$ sieve aboard ship, and material caught on the sieve was preserved for more detailed sieve analysis in the laboratory in Denver. Suspensions of fine sediment that passed the $53\text{-}\mu\text{m}$ sieve were mostly filtered aboard ship onto preweighed Millipore filters (nominal pore diameter $0.45\ \mu\text{m}$) for later weighing in Denver. However, some suspensions of particles finer than $53\ \mu\text{m}$ were bottled and returned to the US for detailed pipette analysis of the silt and clay sizes. Shipboard pipette analyses made in 1976 used river water without added dispersants. Pipette analyses made in the US in 1977 used distilled water and dispersing agents⁶.

Figure 1 compares concentrations of fine-grained (less than $53\ \mu\text{m}$) suspended sediment collected within 1 m of the water surface with the concentrations collected at the same sites from the full depth of water by the depth-integrating sampler. Samples represented in Fig. 1 were collected at six cross-sections spaced along 3,000 km of the Amazon mainstream between Iquitos, Peru, and Óbidos, Brazil. In general, concentration of fine material in suspension at water surface is about half the average fine-material concentration in the full depth of the river. This does not support Gibbs' report⁷ that samples collected at 0.9 total depth (0.1 of the distance from river bed to water surface) in the Amazon had only a 20% greater concentration of suspended sediment than samples collected from river surface at the same sites. Samples taken at 0.9 total depth should have even greater concentration than depth-integrated average concentrations shown in Fig. 1. Furthermore, when total suspended concentration is considered (that is, when material $>53\ \mu\text{m}$ is included), the difference between near-surface and near-bottom concentrations is even greater⁴.

Is there a minimum particle size below which concentration in suspension from bed to river surface is uniform? Figure 2 shows the results of size analyses of samples taken at three different localities with three different types of equipment. The analyses show that particles even as small as $10\ \mu\text{m}$ are not uniformly distributed. In two of the three sets of analyses shown in Fig. 2, concentrations of particles as small as 1 or $2\ \mu\text{m}$ show slight but discernible differences between bottom and surface.

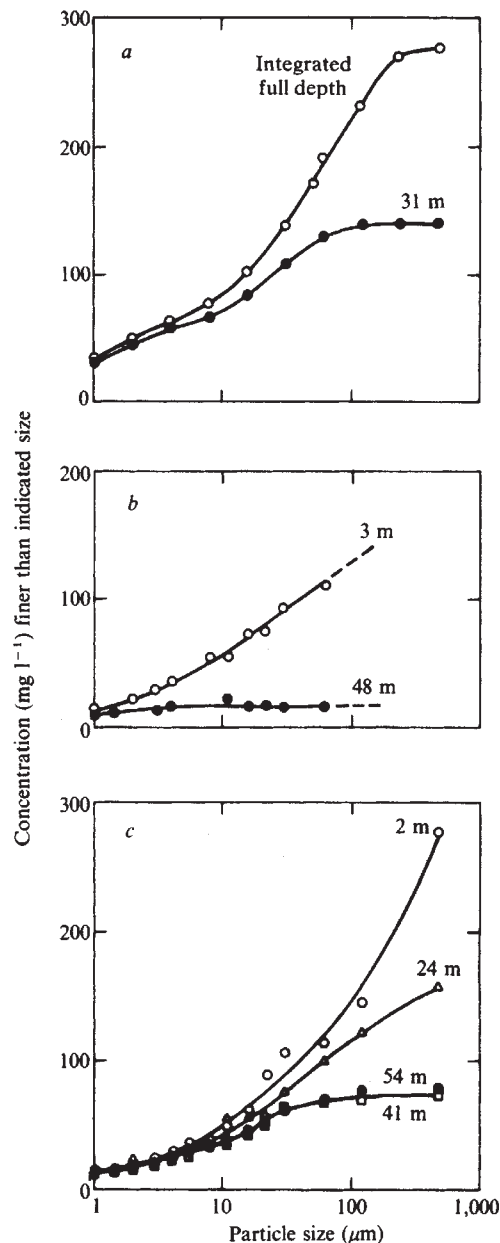


Fig. 2 Particle-size distributions of suspended sediment collected by different sampling methods in the Amazon River. Numbers by each curve indicate distances of samples above the river bed. *a*, Samples collected by depth-integrating sampler through the full depth of the river (from surface to bed and back to surface) and by the integrating sampler at a single depth of 1 m below surface (31 m off river bed). Each curve represents a composite of two samples taken in left centre part of the channel at São Paulo de Olivença, 22 May 1977. *b*, Samples collected by 30-l Niskin bottles 3 m above river bed and at river surface (48 m off river bed) in centre of channel near Manacapuru, 22 June 1976. *c*, Samples collected by point sampler at 2 m, 24 m, 41 m, and 54 m above river bed in left centre part of channel at Óbidos, 14 June 1976. Particle-size distributions at 41 m and 54 m were not sufficiently different to warrant separate lines through their points. Uppermost sample (54 m above the bed) was taken at a depth of 9 m below water surface.

We conclude that, in a river as deep as the Amazon, one cannot assume that suspended particles coarser than $1\ \mu\text{m}$ are uniformly distributed in suspension. In the Amazon, as well as in other large rivers of the world, estimates of sediment yield and flux of suspended chemical constituents that are based only on surface samples are likely to contain significantly large errors.

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Palaeomagnetism of stalagmite deposits

VARIOUS sedimentary deposits have recently been studied with the aim of producing geomagnetic field curves representing the Brunhes epoch. Unfortunately the magnetic recording process tends to be subject to several depositional and post-depositional errors and in the light of these Watkins¹, Verosub² and Verosub and Banerjee³ have reviewed many of the claims that have been made for secular variation and anomalous field behaviour. The corresponding age data, provided by ¹⁴C, uranium series disequilibrium and other methods may also suffer from such errors as bioturbation and migration of radionuclides. It has been shown that most speleothems (here stalagmites and flowstones) may be dated by uranium series methods to an age limit of 350,000 yr BP⁴. With the aim of finding an alternative continuous magnetic record we have studied the natural remanent magnetism (NRM) and ages of speleothems from the Canadian Rockies, the UK and elsewhere. We report here briefly on two of them and in greater detail on a third.

Each speleothem was oriented in the cave using mounts carrying a compass and a clinometer. The sample was cast in

plaster of Paris in the laboratory where it was then sawn parallel and perpendicular to growth horizons, usually into 2-cm cubes. The procedure consisted of measuring the NRM, carrying out a pilot alternating field demagnetisation and then remeasuring the specimens, alternating field cleaned to an optimum level. Fisher statistics were used to find a mean direction for all specimens from the same growth horizon and this direction was then reoriented from its laboratory to its site value.

A flowstone BJTL, 7 cm thick, was collected from Bear Jaw cave near Crow's Nest Pass, Alberta, Canada (Table 1). ²³⁰Th/²³⁴U ratios obtained for the upper growth horizons showed that it was older than 350 Kyr BP (Table 2). Nevertheless, the magnetic results were encouraging in that (1) the major part of the NRM appeared to be stable, the median demagnetising fields of four test specimens all being greater than 180 Oe (Fig. 1 specimen BJTL A2T), with little shift of the concurrent vector direction; (2) the declination (*D*) and inclination (*I*) values were apparently not influenced by surface effects (the flowstone growth horizons dipped at about 28.5° in a direction of 298°). The magnetic intensity ranged from 3×10^{-9} to 2×10^{-8} emu g⁻¹ (the Toronto ScT magnetometer used here has a quoted sensitivity of 2×10^{-7} emu).

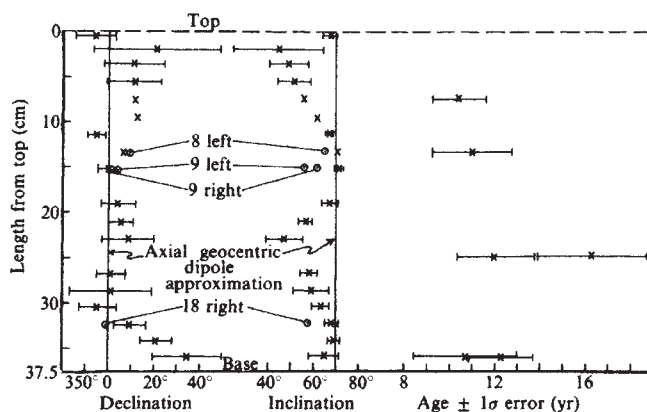


Fig. 2 Declination, inclination and dates of the Kingsdale stalagmite TS. (Direction errors of the lateral specimens have been omitted for clarity.)

A 13-cm thick flowstone from Eagle Cave, Crow's Nest Pass, was reversely magnetised throughout and together with its radioisotope ratios, this suggests that it grew near the end of the Matuyama epoch.

Several speleothems collected subsequently showed very low magnetic intensities. Where the signal was low but the growth layers well defined, greater signal strength was obtained by stacking adjacent specimens of the same growth horizon (suggested by M. L. Glasser). Such was the case of sample TS, a 37-cm high stalagmite from Kingsdale, Yorkshire, UK. Three vertical slices were obtained from the centre of the stalagmite and specimens from the middle cuts were stacked in two's and three's for NRM measurement (Table 1 and Fig. 2). Visual examination of the sample indicated continuous growth from the base, possible flood events having occurred at the levels of cuts 18 and 19, where there is dark opaque detrital material containing quartz grains. Because of their higher intensity, specimens from these lower cuts did not need to be stacked for measurement. Between 1 and 2 cm from the top, at cut 2, there is a concave upwards layer and irregular lateral crystal growth which probably indicates dissolution and hence a growth hiatus. At the time of collection the upper surface was wet, indicating that the top centimetre was probably of recent origin.

Figure 1 shows the alternating field demagnetisation of specimen TS B18Q; the median demagnetisation field was about 400 Oe and the direction moved only slightly from *D* = 6.4°, *I* = 60.0°, to settle around *D* = 10° and *I* = 63°. In view of

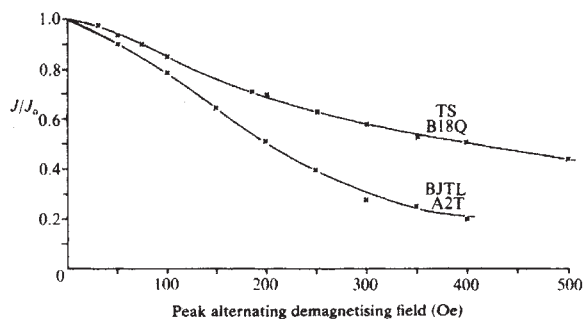


Fig. 1 Specimen demagnetisation curves, where J/J_0 is the ratio of the cleaned magnetisation to the NRM.

Table 1 NRM of samples and analyses of directions

Sample	Cut no.	Distance from top (cm)	NRM					Alternating field demagnetised						
			N	D	I	α_{95}	K	H (Oe)	D	I	α_{95}	K	δD^*	δI^*
BJTL Bear Jaw Cave	1	1.0	9	38.0	77.2	10.6	13	50	42.0	80.5	2.3	410	14	2.3
	2	2.9	10	355.7	75.2	12.4	11	100	10.9	78.1	3.1	200	15	3.1
	3	4.8	9	357.7	69.7	2.5	347	100	355.8	72.0	1.2	1570	3.8	1.2
	4	6.8	8	5.1	65.3	1.5	1072	100	7.3	67.1	1.1	2011	2.8	1.0
TS Kingsdale	1	0.5	3	3.5	64.0	7.3	121	100	354.0	67.2	3.5	529	9.0	3.5
	2	1.9	3	11.3	58.0	32	6.5	100	21.0	44.3	20.2	16	28.2	20.2
	3	3.7	6	355.2	49.7	120	120	50	10.8	49.0	8.3	47	12.6	8.3
	4‡	5.65	4	12.3	44.1	11	37	50	11.1	51.5	7.4	90	11.9	7.4
	5†	7.55	1	14.8	47.5	—	—	50	11.2	55.5	—	—	—	—
	6†	9.55	1	14.8	53.4	—	—	50	12.1	61.8	—	—	—	—
	7‡	11.4	4	355.6	63.0	1.6	2010	50	354.5	66.9	1.7	1670	4.3	1.7
	8†	13.45	1	11.5	63.5	—	—	50	6.4	70.6	—	—	—	—
	9‡	15.2	4	357.7	70.5	1.5	2156	50	359.7	71.2	1.7	1737	5.3	1.7
	10	17.2	Missing											
	11§	19.15	4	(2) 356.5	40.6	16.6	18	50	3.7	66.9	3.4	850	8.7	3.4
	12‡	21.15	4	3.1	56.2	2.5	779	50	5.1	56.5	2.9	583	5.3	2.9
	13‡	23.0	4	10.1	46.0	8.9	62	50	8.8	47.0	8.1	76	11.9	8.1
	14	24.85	Missing											
	15	26.85	3	6.0	55.7	4.9	271	100	0.7	58.3	3.2	626	6.1	3.2
	16	28.7	3	5.2	55.3	9.0	80	100	1.1	59.0	7.9	104	15.3	7.9
	17	30.55	3	3.3	61.5	6.4	158	100	355.4	63.3	3.6	496	8.0	3.6
	18¶	32.4	4	10.0	65.7	3.6	380	100	9.3	67.7	2.7	661	7.1	2.7
	19	34.2	3	20.8	67.2	2.4	1090	100	21.0	69.4	2.7	925	7.7	2.7
	20	35.85	3	35.2	64.3	5.0	263	100	34.7	65.0	6.7	148	15.9	6.7
TS lateral specimens	8‡ Left face	13.45	4	0.0	62.9	2.7	678	50	9.7	63.7	1.5	2227	3.4	1.5
	9‡ Left face	15.2	4	335.7	39.4	22.5	9.7	50	3.5	55.6	5.4	168	9.6	5.4
	9‡ Right face	15.2	4	12.0	59.6	1.5	2053	50	1.3	61.8	2.6	709	5.5	2.6
	18 Right face	32.4	3	0.9	58.3	3.3	618	80	358.6	57.4	2.2	1317	4.1	2.2

* Errors from $\alpha_{95} = \cos I \delta D = \delta I$.

† Two specimens stacked to give one vector.

‡ Three specimens stacked in two's and then all three together to give four vectors.

§ B11Q possessed an anomalous cleaned direction of 353° and -32° after vector subtraction and has therefore been excluded from the alternating field demagnetised mean. We do not know the reason for this.

|| Cuts 10 and 14 were not measured because of extensive shattering incurred during the casting process. B14Q and D14Q have been used for dating purposes.

¶ Cut 18 also included specimen D19R which had exactly the same growth horizons.

the low intensities of the middle specimens it was felt that 50 Oe was an optimum demagnetising field. For most cuts the precision has increased on cleaning.

If speleothems possessed some kind of detrital remanent magnetism (DRM) the surface of deposition would be expected to have had an effect on the NRM directions. To test for this, specimens were prepared from the vertical sides of the Kingsdale stalagmite. They were taken from cuts 8 left-, 9 left-, 9 right- and 18 right-facing sides and contain, more or less, horizons spanning cuts 1 to 7, 8, 8 and 17 respectively. Their values (Table 1 and Fig. 2) are in accordance with other values for these layers, strongly suggesting that neither the D nor I values have been influenced by surface effects. This in turn points to a remanence of chemical origin.

The speleothem specimens have been dated by the $^{230}\text{Th}/^{234}\text{U}$ method assuming that no ^{230}Th was initially present and that the system had remained closed after initial deposition of the

uranium. (Detrital thorium contained in the soluble fraction is detectable by the presence of ^{232}Th in the α -particle spectrum. Our method also takes into account the excess of ^{234}U over ^{238}U .) Radiometric data for TS (Table 2 and Fig. 2) show that it is young; the low uranium content renders the data imprecise. The stalagmite appears to have grown from about 12,500 yr BP, and was later redissolved down to an horizon which grew at about 10,000 yr BP, the remaining cap having been deposited more recently. Thus between 12.5 and 10 kyr BP it grew at 20 cm kyr $^{-1}$.

To test whether the stable primary magnetisation was syngenetic with its host growth horizon we have compared the modern field direction of the Kingsdale area with that for the upper cap of the Kingsdale stalagmite. (This is in contrast to many lake or marine cores where the tops are either missing or are severely disturbed. Note also that NRM direction errors, especially of inclination, may often go undetected in such cores.)

Table 2 Specimen dating and isotope activity ratios

Sample	Specimen	U (p.p.m.)	$^{230}\text{Th}/^{234}\text{U}$	$^{234}\text{U}/^{238}\text{U}^*$	$^{230}\text{Th}/^{232}\text{Th}$	Calculated age $\pm 1\sigma$ error (kyr BP)	Distance from top to 0.1 cm
Bear Jaw BJTL	1C	1.1	0.98 ± 0.04	1.00 ± 0.04	609	> 350	0.9
Eagle cave ENF	A1	0.27	1.10 ± 0.05	1.26 ± 0.05	17	> 350	0.9
Kingsdale TS	B5Q	0.15	0.092 ± 0.01	1.68 ± 0.085	9.9	10.4 ± 1.2	7.55
	B8Q	0.13	0.097 ± 0.015	1.70 ± 0.08	3.5	11.0 ± 1.8	13.45
	B14Q	0.12	0.14 ± 0.02	1.70 ± 0.08	> 1000	16.3 ± 2.4	24.85
	D14Q	0.16	0.11 ± 0.01	1.77 ± 0.06	> 1000	12.0 ± 1.7	24.9
	D20Q	0.16	0.09 ± 0.02	1.61 ± 0.06	14.5	10.7 ± 2.3	35.8
	E20Q	0.18	0.11 ± 0.01	1.61 ± 0.06	41	12.3 ± 1.4	36.0

* Low ^{232}Th count rates indicated the essential lack of detrital thorium in the Kingsdale TS sample.

The modern field direction is $D = 8^\circ\text{W}$, $I = 69^\circ$, while the three specimens of cut 1 which was only 1 cm in depth have a mean direction of $D = 6^\circ\text{W}$ and $I = 67^\circ$ with an α_{95} of 3.5° ; this is probably an average of the past 50 yr or more.

Of nine other speleothems which we have analysed, two are too weakly magnetised to be measurable, two show large viscous instabilities, and the remainder have stable, measurable NRM's. The latter, like the Bear Jaw and Kingsdale samples, have NRM's apparently unaffected by surface forces such as roughness, water flow, crystal growth habit or the dip of the growth layers, and this again supports the idea of a chemical remanent magnetisation. Hysteresis curves obtained for several specimens show saturation fields of around 3,000 G and coercivities of remanence between 140 and 350 G; this indicates that magnetite is the major carrier of the remanence.⁵

Although the stable remanence of speleothems is often weak, our findings indicate that in many cases we will be able to produce good records of secular variation in D and I . We expect the uranium concentrations will be sufficient to allow us to assign accurate absolute ages to most of these records.

A disadvantage of this method is that speleothems are objects of great natural beauty which once removed from a cave cannot be replaced. It is better to investigate limestone quarries where caves are sometimes breached and where speleothems may in any case be destroyed.

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Growth rate of a clam from the Galapagos Rise hot spring field using natural radionuclide ratios

THE submarine hydrothermal springs on the Galapagos Rift are the sites of lush biological populations supported ultimately by sulphur-oxidising bacteria utilising hydrogen sulphide transported from depth by the springs¹. Two types of bivalves, one a mytilid mussel and the other a vesicomyid, have been recovered from these thermal regions. The shells of these organisms are as much as 30 cm long. We are determining growth rates of these organisms using a method which measures natural radionuclide content². In this report we present our first results on a clam collected in the February–March 1977 Alvin dive series.

A 22 cm long valve of an articulated but dead specimen of a vesicomyid clam from the active thermal spring area, called Clambake I¹, at a depth of about 2,500 m, was analysed for the radionuclides listed in Table 1 within 1 yr after collection. The valve was cut in half from the hinge to the growing edge. The analyses were made on aliquots of the pulverised and homogenised half with the other half remaining intact for growth structure analysis and other studies. The analytical procedures involve α -spectrometric techniques for the metallic radionuclides (except ^{226}Ra which was determined by scintillation counting of ^{222}Rn)³, and liquid scintillation analysis for radio-carbon assay⁴.

Using the data shown in Table 1 we can first set upper limits to the age of the clam by making limiting assumptions about the radiochemical composition of the water from which the clam deposited its shell. In all of these calculations we assume a constant mass deposition per unit time growth law. We do not yet know the exact growth law for these deep-sea clams but shallow water clams generally show the following pattern⁵: an initial slow growth followed by a quasi-linear mass increase with time and then a slowing down at old age. By assuming that at all times in the life of the Galapagos Rift vesicomyid it deposits shell at a constant mass rate we will be erring in the direction of calculating too old an age.

The lowest $\delta^{14}\text{C}$ value for dissolved inorganic carbon at depth in the eastern South Pacific measured in the GEOSECS programme by Ostlund *et al.*⁶ is -240 . If the shell grew with a constant mass per unit time, then its age can be calculated by the equation: $A/A_0 = (1 - e^{-\lambda t})/\lambda t$, where A is the activity after time t and A_0 is the initial activity. The calculated age by this model is about 500 yr. The assumption that the water from which the shell grew is unmodified ambient Pacific deep water conflicts with the observations¹. Indeed a significant amount of dissolved inorganic carbon is released by the vents to the bottom water. This carbon can only be lower in ^{14}C than the ambient dissolved carbon whether it is imprinted by mantle material or old calcium carbonate deposits on the ridge flanks.

The $^{210}\text{Pb}/^{226}\text{Ra}$ activity ratio in the shell is 3.8. Without independent information of the $^{210}\text{Pb}/^{226}\text{Ra}$ ratio of the bottom water in this region an age assignment cannot be made. We proceed as follows. If the ^{226}Ra activity is 60 d.p.m. per 100 kg (P. E. Biscaye, personal communication) and the ^{222}Rn activity is 6,000 d.p.m. per 100 kg (J. Dymond and J. Edmond, personal communication), then in steady state conditions, with the ^{210}Pb activity equalling the ^{222}Rn activity, the expected initial activity ratio of $^{210}\text{Pb}/^{226}\text{Ra}$ would be about 100 in the water. If no strong fractionation between ^{210}Pb and ^{226}Ra occurred during shell deposition⁷ this would also be the initial ratio in the shell. A constant growth model would then yield an age of 830 yr. It is unlikely that the ^{210}Pb produced from the radon would persist in solution, however. Rather it will be scavenged by the precipitating ferromanganese oxides found everywhere in the region

Table 1 Radiochemical analyses of a vesicomyid clam from the Galapagos Rift (Clambake I)

Activities (d.p.m. g ⁻¹)	
^{226}Ra	0.0515 ± 0.0022
^{228}Ra	0.00200 ± 0.00029
^{228}Th	0.00135 ± 0.00030
^{210}Pb	0.195 ± 0.006
Activity ratio	
$^{234}\text{U}/^{238}\text{U}$	1.19 ± 0.08
$^{228}\text{Th}/^{228}\text{Ra}$	0.68 ± 0.18
$^{228}\text{Ra}/^{226}\text{Ra}$	0.039 ± 0.006
$^{210}\text{Pb}/^{226}\text{Ra}$	3.79 ± 0.20
$\delta^{14}\text{C}$	-213 ± 8
$\Delta^{14}\text{C}$	-263 ± 10

All measurements are corrected to time of collection. Sample is designated G3-2(L) obtained on drive 713 (approximately $0^\circ 47.6' \text{N}$, $86^\circ 09' \text{W}$).

including on the shells of living mussels. As the estimated ^{210}Pb value in the water from which the shell is growing is diminished the calculated age of the clam will also become smaller.

The $^{228}\text{Ra}/^{226}\text{Ra}$ activity ratio of the shell provides a third method of setting an upper limit. This will, however, be more constraining than the previous two methods since two isotopes of the same element are involved and the limits on the ratio of the two isotopes in the water can be more accurately defined. An upper limit to the $^{228}\text{Ra}/^{226}\text{Ra}$ ratio in the water is set by the ratio of $^{232}\text{Th}/^{238}\text{U}$ in the basalt undergoing leaching. Mid-ocean ridge basalts have a Th/U weight ratio of around 2 (ref. 8). This corresponds, at secular equilibrium, to a $^{228}\text{Ra}/^{226}\text{Ra}$ activity ratio of about 0.65. If this were the ratio for the water from which the clam deposited its shell, the constant mass growth law age would be 140 yr.

The only direct method of arriving at the growth rate of the clam is by the use of the $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio. ^{228}Ra is supplied to the water at the ocean bottom from the ocean floor rocks and sediments as the result of production by radioactive decay of ^{232}Th and diffusion. Once in the water ^{228}Ra decays with a half life of 5.7 yr to ^{228}Th which decays with a half life of 1.9 yr. As the shell grows it adds ^{228}Ra , presumably with little or no ^{228}Th , just as it adds ^{226}Ra . As the deposited shell material ages, ^{228}Ra decays within the shell to ^{228}Th which itself is subject to radioactive decay. Using our assumption that the rate of precipitation of calcium carbonate by the organism is constant with time during its life, the average $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio of the whole shell will be a unique indicator of its age at time of capture. The relevant equation is:

$$\frac{A_T}{A_R} = \frac{\lambda_T}{\lambda_T - \lambda_R} \left[1 - \frac{\lambda_R(e^{-\lambda_T t} - 1)}{\lambda_T(e^{-\lambda_R t} - 1)} \right]$$

where R and T refer to ^{228}Ra and ^{228}Th respectively and A values are activities.

Figure 1 shows the plot of $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio for the whole shell as a function of age of organism assuming constant rate of calcium carbonate deposition. From Table 1 the measured ratio in our clam is 0.68 ± 0.18 , where the stated error is the 1σ counting error. This ratio corresponds to an age of 6.5 yr. At the 1σ level the uncertainty is $-3, +5$ yr.

This means that the growth rate of the long axis of this clam is about 4 cm yr^{-1} and the mass growth rate per animal (both valves) is about 20 g CaCO_3 per yr. We compare this with the growth rate we reported for an Atlantic Ocean deep-sea clam, *Tindaria callistiformis*², which reaches a length of 8.4 mm in about 100 yr for an average growth rate of $0.0084 \text{ cm yr}^{-1}$; about 500 times slower. Evidently the opportunistic nature of the clams living on the peculiar food source at the thermal vent permits a more rapid growth than the general ambient deep ocean floor environment. The same sort of opportunism has been reported for the deep ocean wood-boring clam, *Xylophaga* sp. by Turner⁹. She reported that 2 cm thick wooden planks, inserted in the North Atlantic ocean floor at a depth of 1,800 m by submersible and subsequently recovered, were almost completely devoured in 100 d by these clams.

Growth rates of clams on the deep ocean floor then appear to be primarily a function of the rate of food supply.

If we accept an age of 6.5 yr for this clam then we can interpret the radiochemical data used in establishing the upper age limits discussed above.

The $\Delta^{14}\text{C}$ of the clam is about 10% lower than the expected value from Pacific Deep Water. This can be ascribed to dilution of the deep water with old carbon supplied from the vents. Corliss *et al.*¹ determined the slope of the dissolved inorganic carbon to heat (via temperature) at $25 \times 10^{-9} \text{ mol cal}^{-1}$. A temperature of 12°C for the water in which the clam grew corresponds to an increase of total inorganic carbon of $250 \mu\text{mol kg}^{-1}$ above the Pacific Deep Ocean value of about $2,400 \mu\text{mol kg}^{-1}$ (ref. 10) (at a temperature of 2°C). This is an approximately 10% increase which matches the observed ^{14}C decrease, if the vented carbon were totally devoid of ^{14}C .

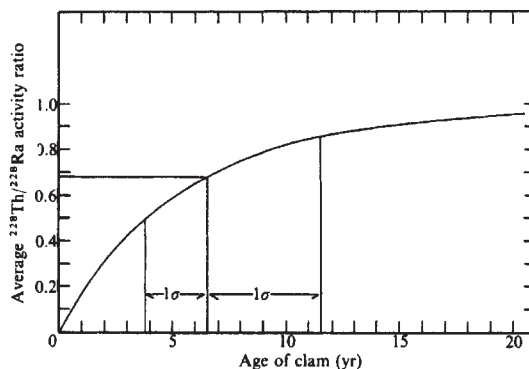


Fig. 1 The change in the average $^{228}\text{Th}/^{228}\text{Ra}$ activity ratio in a clam depositing a constant mass of calcium carbonate per unit time with a constant $^{228}\text{Ra}/\text{Ca}$ ratio and no initial ^{228}Th . A vesicomyid clam (Dive 713, Sample G3-2(L)) collected dead, but articulated, has an age at time of collection of 6.5 yr on the basis of this diagram.

Lead-210 appears to be sequestered by shallow water calcareous organisms more efficiently than radium by a factor of no more than two⁷. The $^{210}\text{Pb}/^{226}\text{Ra}$ ratio reported in Table 1 may then be assumed to be equal to or greater than the ratio in the ambient water in which the clam grew. As the shell is about 6.5 yr old, only a small amount of ^{210}Pb decay will have occurred, and the ratio provides a measure of the $^{210}\text{Pb}/^{226}\text{Ra}$ ratio in the water in which the clam grew. The deficiency of ^{210}Pb over that expected from the production from dissolved ^{222}Rn then provides a measure of the efficiency of the chemical removal process for ^{210}Pb from seawater in the region of the clam's habitat. The following material balance calculation can be made assuming a first order law for chemical removal as well as radioactive decay: Activity of ^{222}Rn (A_{Rn}) = Production rate of ^{210}Pb = Rate of radioactive decay of ^{210}Pb (A_{Pb}) + Rate of chemical removal of ^{210}Pb . Therefore:

$$A_{\text{Rn}} = A_{\text{Pb}} \left(1 + \frac{\tau_{\text{Pb}}}{\tau_c} \right)$$

where τ_{Pb} (32 yr) and τ_c are the mean life of ^{210}Pb and the chemical removal residence time respectively. Referring to our calculation above involving ^{226}Ra and ^{210}Pb and using the same normalisation we obtain, as an upper limit, assuming equal fractionation of ^{210}Pb and ^{226}Ra :

$$\tau_c = \frac{32}{26-1} = 1.3 \text{ yr}$$

This implies that scavenging of ^{210}Pb is proceeding efficiently at least in the rock-water interface where the clam is growing. Corliss *et al.*¹ have already indicated how effectively seawater is stripped of Cu, Zn, Cd, Ni and U as the result of precipitation by H_2S within the "plumbing" of the thermal vent even before massive dilution with seawater occurs. Our results indicate that scavenging of ^{210}Pb , and possibly certain other elements that behave the same chemically, occurs even in the mixing zone between the hot water coming through the complex plumbing in the basalt and the ambient cold seawater of the ocean bottom. This is probably in the major part due to precipitation of ferromanganese oxides.

The initial $^{228}\text{Ra}/^{226}\text{Ra}$ value (0.057) in the clam is of the same order of magnitude as the ratio found in bottom water at GEOSECS station 263 from the South Pacific¹¹. This concordance, however, may be largely fortuitous. If half of the radium in the bottom-most water in which the clam grew were derived from the leaching of basaltic rocks, the $^{228}\text{Ra}/^{226}\text{Ra}$ activity ratio of the bottom water would be about 0.34 which is markedly higher than the predicted value from the shell. In order to attain the measured value the length of time necessary to transfer the radium from its source to the surface would have to be 15 yr to

allow sufficient decay time for ^{228}Ra . On the other hand the high radon standing crop (6,000 d.p.m. per 100 kg) implies derivation from a secondary deposit of ^{226}Ra near the water-rock boundary. We cannot therefore identify the exact sources of the two radium isotopes based on the shell analysis.

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Eucalyptus fruits in stratigraphic context in Australia

THE eucalypts are the outstanding group of *Leptospermeae* (subfamily *Leptospermoideae* of *Myrtaceae*) and of the whole Australian flora¹. There are over 500 species of eucalypts, nearly all restricted to Australia. The origin of this remarkably diverse and successful genus has been a matter for speculation. The comparatively well known Tertiary palynological record is of little assistance in answering these questions, as spores and pollen have insufficient properties to identify the genus. Hence

only the macrofloral record is taxonomically significant. Although Tertiary macrofossils⁴ are abundant, the fossil record of *Eucalyptus* and even *Leptospermoideae* is poorly known^{2,3}. The first unequivocal fossil gumnuts (*Eucalyptus* fruits), of indefinite age, were identified only recently from silicified floras near Woomera⁵, in South Australia's present arid zone. These specimens were collected without reference to the stratigraphy. However, we have now collected specimens from similar silicified outcrops near Stuart Creek (Fig. 1), where their probable age may be deduced by correlation of the sediments with Tertiary sequences elsewhere in South Australia. *Eucalyptus* can now be placed in stratigraphic context.

Although Tertiary stratigraphy in South Australia is in a state of flux^{7–12}, we have been able to restrict the age range of the fossils discussed here (Fig. 2) to two possibilities within the Tertiary. The alternative correlations presented here permit an Eocene–Oligocene age on the one hand, and Miocene age on the other. All that could have been said previously was that the gumnuts were Mesozoic or younger.

We investigated the site in October 1978, and Fig. 2 shows a selection of gumnuts from the fossiliferous sediments. Other fruits and leaves present in abundance will be described elsewhere. The flora was collected from a sandstone-conglomerate sequence (Fig. 3, section 1) which disconformably overlies Aptian–Albian shales⁶. The basal conglomerate has clasts of quartzite, kaolinised shale, cream and grey cryptocrystalline silcrete and polished chert, jasper and quartz. Overlying this unit are cross-bedded, mature fine- to medium-grained sandstones. On a nearby ridge (Fig. 3, section 2), similar but unfossiliferous siltstones, sandstones and conglomerates disconformably overlie Cretaceous beds; the upper units here are representative of Tertiary sandstones north and south of Stuart Creek. The plant fossils are in a different facies, but the two sections are thought to belong to the same sedimentary cycle.

The Tertiary of South Australia in the Lake Eyre and Pirie-Torrens Basins, abutting the northeastern and southeastern margins of the area under discussion, has usually been divided into two formations of characteristically distinct lithology. Extensive sheets of coarse, mature sands and silts (Eyre Formation⁷), determined palynologically as Palaeocene to Eocene, are disconformably overlain by the rather immature fine sediments

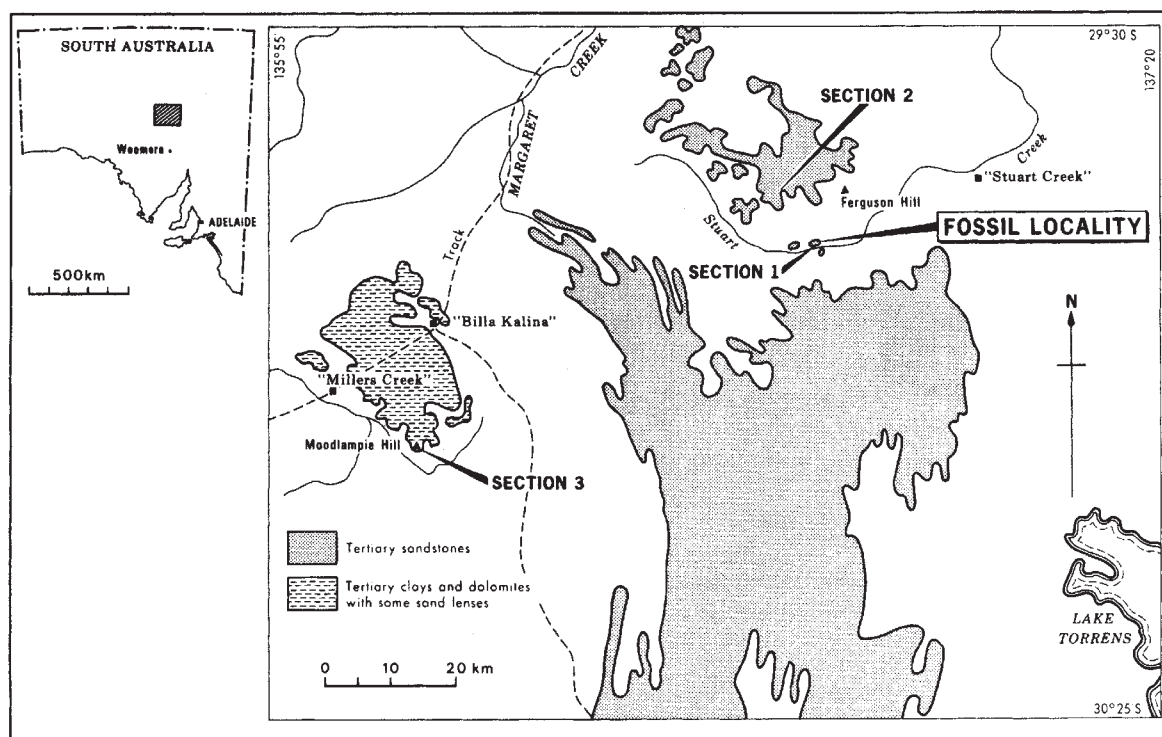


Fig. 1 Simplified Tertiary geology of the Billa Kalina homestead area, showing positions of stratigraphic sections shown in Fig. 3.

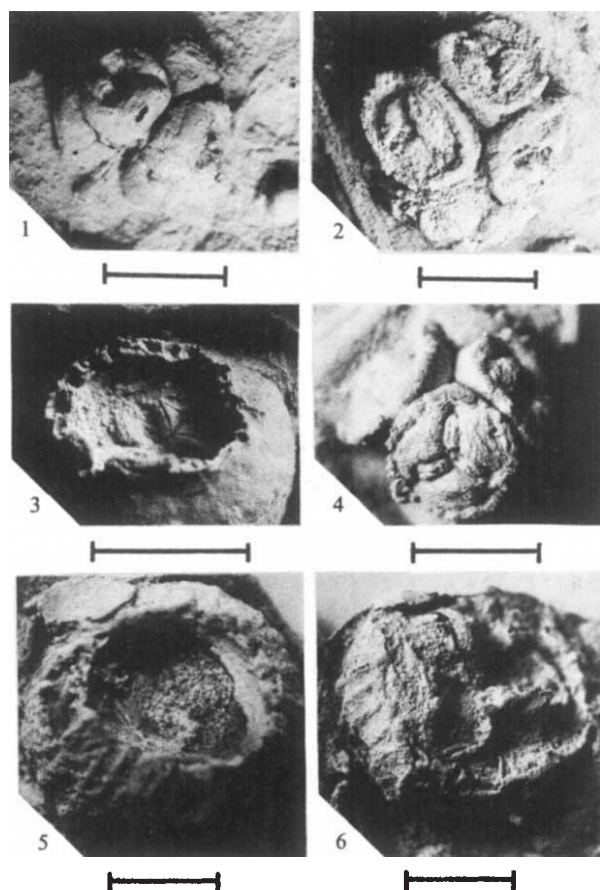


Fig. 2 Gumnut fossils from the Stuart Creek area. Scales represent 5 mm. Specimen 1: inflorescence of seven eucalypt-like capsules nearly sessile on a peduncle, each wedged against its neighbour but free at the summit which is flattened and exhibits three-valved loculicidal dehiscence. Specimens 2, 4: similar but another species, with raised rim, vertical disk and convex capsule dehiscent by three valves between which seed and chaff plugs sometimes remain. Specimens 3, 5: larger solitary fruits consistent with those of eucalypts, of maximum diameters much exceeding orifice diameter; with narrow raised rims, deep vertical disks and sunken capsules. One displays initial traces of three-valved dehiscence. Specimen 6: a ribbed eucalyptoid capsule with a flat summit and wide-gape three-valved dehiscence, the broken valve-tips apparently exerted.

of the almost equally extensive Medial Miocene, characterised by green clays and dolomites (Etadunna Formation⁹). The Medial Miocene age was also determined palynologically^{7,10}, from the basal Etadunna Formation and equivalents. Its upper strata contain vertebrates which are unlikely to be younger than Late Miocene (R. H. Tedford, personal communication 1973) so the unit is essentially Miocene.

The gumnut-bearing conglomerate contains rounded and sorted cryptocrystalline silcrete (siliceous duricrust) pebbles. Despite much mapping and drilling of Tertiary strata throughout South Australia and adjacent regions, no pre-Eocene silcrete of this type is known. Thus Tertiary sediments containing silcrete pebbles are probably Eocene or younger. However, the fossiliferous sediments are lithologically similar to the Eyre Formation in the adjacent basins. They could therefore represent the upper part of the Eyre Formation deposited contemporaneously with silicification, or a separate phase of sedimentation partly synchronous with or younger than this

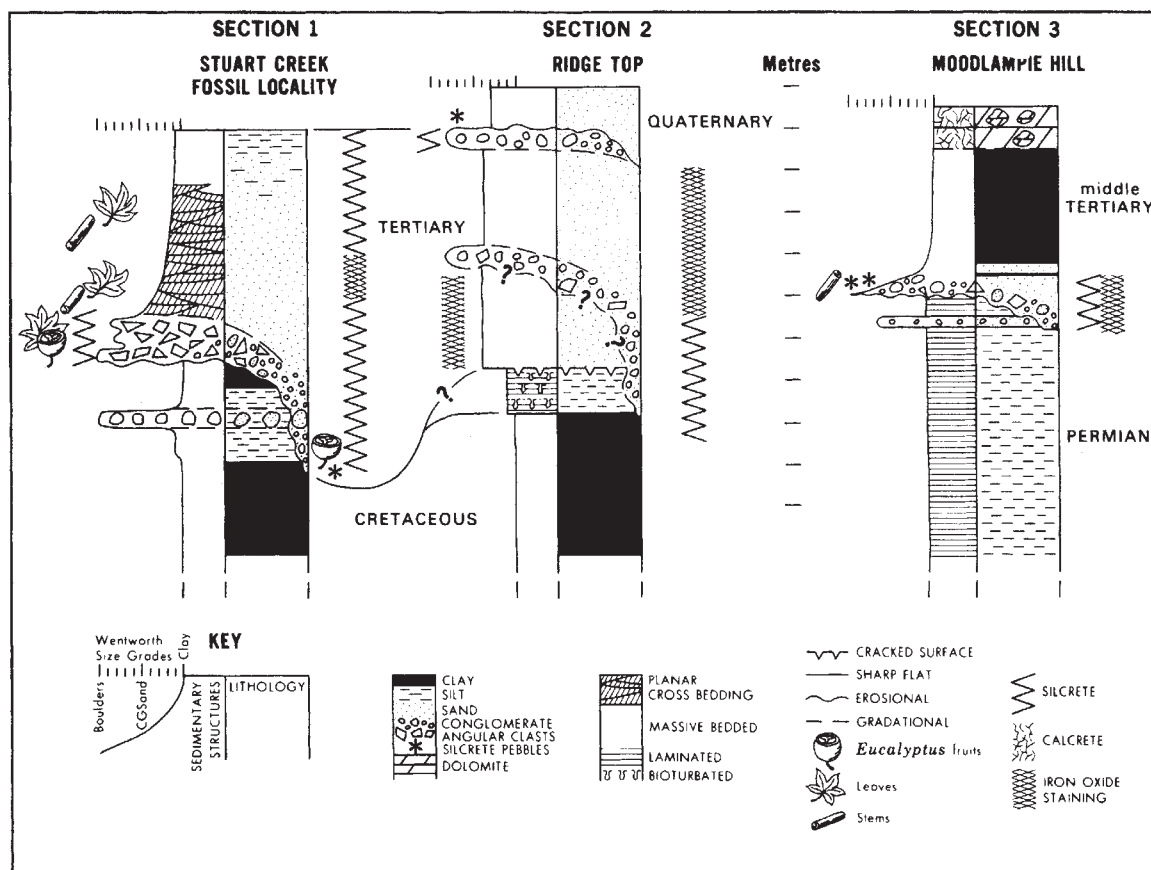


Fig. 3 Key stratigraphic sections. Tertiary sequences are disconformable on Cretaceous and Permian sediments and overlain by Pleistocene sand dunes in section 2.

Formation (of Eocene–Oligocene age) following a major interval of duricrust development.

On the other hand, at Moodlampie Hill (Fig. 1), conglomerate and sandstone intertongue with green clay and carbonate (Fig. 3, section 3) which we correlate with the Etadunna Formation of the Lake Eyre Basin. The conglomerate and sandstone, however, are similar to that at Stuart Creek, containing silcrete pebbles. A stratigraphic analysis of Tertiary sediments in the Billa Kalina area indicates that sediments at Moodlampie Hill and Stuart Creek may be facies equivalents (G.J.A. and R.B.F., in preparation). On this basis the *Eucalyptus*-bearing sediments would be Miocene. This concept presents problems in terms of currently accepted Tertiary stratigraphy^{7,9,10}, but is the simplest explanation in local terms. It opens the possibility of a late Tertiary age for at least part of similar extensive sandstone sheets elsewhere in the state, at present regarded as Eyre Formation.

Australian Palaeogene vegetations represented in southern and eastern near-coastal macro-floras were like those found today in equatorial and tropical wet regions, apparently being devoid of *Leptospermeae*^{14,15}. Such conditions are supported by evidence from marine planktonic organisms, palynological data, and palaeotemperature measurements^{16–19}. Kemp¹⁶ has shown that in the Australian flora (excluding *Eucalyptus* which is not mentioned) there is no evidence of pronounced cooling until the Oligocene, and none of open vegetation reflecting seasonal aridity until the Miocene. Climatic zonation is apparently absent for the Eocene, but floral data suitable for reconstructing climatic zones are only available for this epoch, and even then derive mainly from southern Australia and the present coastal regions. By analogy with Quaternary climatic events one expects dry conditions and a zonal climatic distribution to have coincided with the periods of pronounced cooling in the late Eocene–Oligocene, and late Miocene. Hence xeromorphic vegetation and *Eucalyptus* in particular is unlikely to have radiated until at least the very late Eocene times. Xeromorphy, however, may have originated earlier, and there is some evidence for xeromorphy and cooling in the middle Eocene¹⁶. This is in keeping with the alternative ages suggested for the flora. In this context, the diversity of species in the silcrete floras will also be significant.

The gumnuts should have been readily preserved in the abundant coastal early Tertiary macrofloras, had they been even a minor component of the source litter. If they are Miocene or Oligocene, their absence from these Palaeocene–Eocene floras is readily explained, and their age is in accord with climatic interpretations¹⁰. If they are early to middle Eocene, then in Central Australia conditions must already have deteriorated from absolute mesicity, permitting upsurge of xeromorphic *Leptospermeae* so as to displace more mesic vegetation coastwards. This may imply drier conditions, suggesting rifting from Antarctica was sufficient to produce climatic changes influencing Australia, before the major cooling episode of the late Eocene to Oligocene.

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Long-term recognition of father's song by female zebra finches

RECENTLY, attention has been directed at the question of how sexual imprinting, or the development of mating preferences, affects assortative mating within polymorphic species^{1–3} and regulates the extent of inbreeding and outbreeding in a population^{4–6}. It may be selectively advantageous for a young organism to learn individual characteristics of parents and/or siblings to avoid subsequent mating with kin as well as to avoid hybridisation by not mating with individuals that differ greatly from rearing partners^{4–6}. I report here that adult female zebra finches (*Taeniopygia guttata*) can recognise the song of their own father after a period of early exposure followed by more than 2 months of separation while attaining sexual maturity.

The zebra finch is an Australian grassfinch (family Estrildidae), the young of which are totally dependent on parental care on hatching. Hatchlings are fed by both parents and do not leave the nest until about the third week⁷. Between days 30 and 40, the young leave (or are driven off by) the parents and form mixed flocks with other young finches. Sexual maturity is reached by 90 d (ref. 8). Thus, in early development (that is, before 40 d of age), females are exposed to the song of their father, and thereafter to the developing songs of male siblings that typically bear some resemblance to the father's song^{9,10}. Adult male zebra finch song is highly variable between individuals in acoustic structure but relatively stable within an individual, thus providing a possible basis for individual recognition¹¹.

Females were hatched in rooms containing 12–21 breeding cages (82.0 cm long, 39.0 cm high, 30.0 cm deep) with one adult zebra finch pair per cage. (No other species were housed in these rooms.) At 35 ± 3 d after hatching, females were separated from their parents and housed in a soundproof room with other young female zebra finches in 82.0 × 39.0 × 30.0-cm cages, each containing three females. At day 100 ± 3, each female was given a single 30-min simultaneous auditory choice test involving the song of her father and either the song of another adult male zebra finch that differed considerably from her father's song in acoustic structure, or the song of another adult male zebra finch that was similar to her father's song in acoustic structure. All male zebra finch songs used in the playback tests were recorded when the male was singing to his mate. No sounds other than the male's song were on the test tapes. Examples of the songs are shown in Fig. 1. The purpose of the first type of test (father's song compared with dissimilar song) was to assess the general capability of a female to recognise and show a preference for her father's song over that of a randomly chosen conspecific male that was not a close relative. The second type of test (father's song compared with similar song) assessed the specificity with which a female could distinguish her father's song from one that

Table 1 Choices of female zebra finches in simultaneous auditory choice tests

Test	n	Percentage responding	No. showing preference	Latency (s)		Duration (s)	
				Mean	s.d.	Mean	s.d.
Father's song compared with dissimilar song	31	77	17	527 ± 616		1,069 ± 746	
			$P < 0.05$	$P < 0.003$		$P < 0.001$	
Father's song compared with similar song	21	86	7	1,242 ± 768		401 ± 661	
			9	637 ± 661		942 ± 696	
			NS	NS		$P < 0.02$	
			4	865 ± 806		503 ± 600	

Statistical comparisons of preference scores were assessed using the χ^2 one-sample test (d.f. = 1), and latency and duration scores were assessed using the Mann-Whitney U test. All probability values are two-tailed. NS, Not significant.

was similar. (Because song structure can differ considerably among individual males, a song similar to that of the father could be obtained only by recording either the female's uncle and/or male siblings, as related males tend to have similar songs due to early vocal learning^{9,10}.) Each female was tested only once in either the first or second test.

The rectangular test apparatus, housed in an anechoic chamber, was divided into two approach zones at opposite ends separated by a neutral zone, each zone being 70.5 cm long. Two loudspeakers were located outside the apparatus adjacent to each approach zone. Behavioural measures recorded were (1) latency of approach from a centrally located start box in the neutral zone to each approach zone, and (2) total time spent in each approach zone. A preference was recorded when a bird spent twice as much time in one approach zone as in the other.

The results are shown in Table 1. Female zebra finches recognised and significantly preferred the song of their own

father to the dissimilar song of another male zebra finch. When confronted with the perceptually more difficult test of the father's song compared with a similar song, females still recognised their father's song. Although the tendency of females actually to prefer their father's song in the latter test was not statistically significant, those females that did prefer their own father's song had significantly longer duration scores than those preferring the similar song.

Thus, female zebra finches can recognise their father's song as a function of early exposure followed by a relatively long period of isolation from male song. These tests of approach behaviour do not necessarily reflect sexual motivation on the part of the female and therefore do not represent a demonstration of sexual imprinting as such. Rather, they demonstrate the extent to which females can recognise and discriminate their father's song from that of another conspecific male. Indeed, as females presumably do not mate with their fathers in nature, I would have predicted the outcome of these tests to be quite different had they been assessing sexual behaviour. Nevertheless, this study may have important ramifications regarding the study of sexual imprinting in general. The learning of both supra-individual (species) and individual characteristics during early exposure may, at the time of sexual maturity, influence an animal to (1) mate with its own species—a direct effect of learning supra-individual features—and at the same time (2) avoid mating with close kin—a direct effect of individual recognition. However, many studies^{12–16} have shown that, in several species, individuals can identify conspecifics in advance of exposure to them and that embryonic and postnatal self-stimulation probably plays an important part in these and other instances of 'instinctive' preferences for species-typical stimuli^{17,18}. Thus, it is conceivable that the role of imprinting in mate selection in nature may be of greater relevance to the learning of individual rather than supra-individual characteristics.

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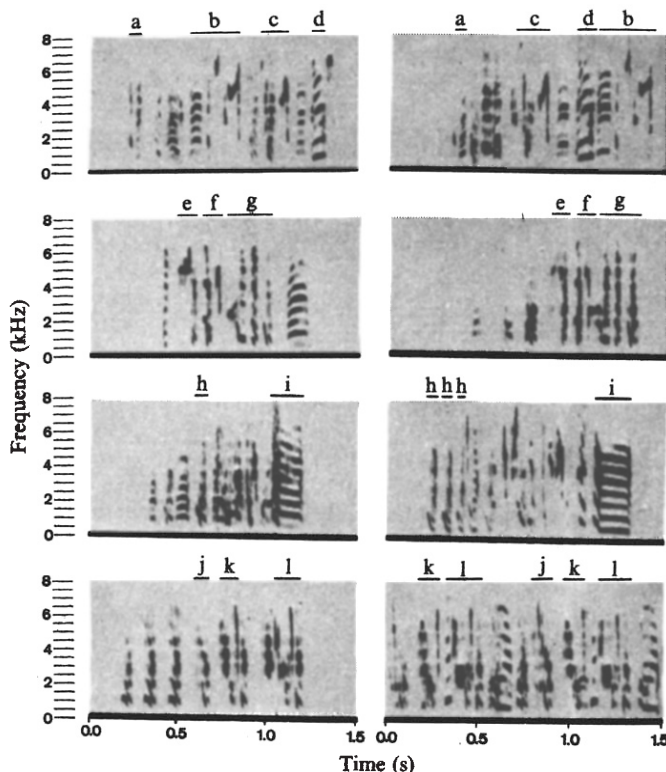


Fig. 1 Wide-band sound spectrograms of song phrases from eight different adult male zebra finches. Each pair (row) of sound spectrograms depicts two songs that are similar to one another and were uttered by two males that were closely related (either brothers or father and son). The common elements in these phrases are depicted above each spectrogram with a bar and a letter. The four spectrograms going down each of the columns are dissimilar from one another in acoustic structure and were uttered by unrelated males.

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Vertebrate and invertebrate predation intensity on freshwater zooplankton communities

PREDATION is widespread in the pelagic zone of lakes and both vertebrate and invertebrate predators are abundant and diverse in their feeding habits. Vertebrate predators are often size-selective and can virtually eliminate larger prey items^{1–3}. These dramatic effects resulting from fish predation have led several workers to conclude that vertebrates contribute the largest percentage of the total predation intensity on freshwater zooplankton. Particular invertebrate predators, however, have also been shown to crop a substantial proportion of their prey populations^{4–6}. I report here a study showing that invertebrate predation is more intense than vertebrate predation in the pelagic zone of Gull Lake, Michigan, with the first *in situ* evaluation of both types of predation on a single zooplankton prey assemblage. The significance of identifying major predation pathways in lakes is basic to many contemporary discussions of nutrient and energy flow as well as considerations of community structure and stability.

Gull Lake is a marl lake in southwestern Michigan which has undergone excessive nutrient enrichment^{7–9}. The lake is 8.2 km² in area and 33 m deep. Following the development of a hypolimnetic oxygen deficit in 1966, there have been large changes in the zooplankton community. The present dominant species are *Bosmina longirostris*, *Cyclops bicuspidatus* and *Tropocyclops prasinus*. There are six species of *Daphnia* and moderate populations of *Diaptomus oregonensis* and *D. minutus*. Large copepods such as *Mesocyclops edax* and *Epischura lacustris* and one predaceous cladoceran (*Leptodora kindtii*) are present but rare. *Chaoborus* spp. are immature dipterans that live in the bottom sediments.

There are over 70 species of fish in Gull Lake; however, most of these are inshore or rare species. In 1952, smelt (*Osmerus mordax*) were introduced into the lake. By 1966, this species had become the dominant fish in the pelagic zone and it now represents 95–100% of all fishes taken with vertical gill nets in the central basin. This species schools by age class and is a stenotherm¹⁰. As eutrophication has progressed, smelt have become stressed, as their requirements for cold water and high oxygen concentration preclude their use of much of the anoxic hypolimnion and the warm epilimnion in summer. Immature fish are sometimes found in the upper few metres of Gull Lake but their appearances are highly seasonal. The population and predation dynamics of juvenile *Lepomis macrochirus* and *Labidesthes sicculus* have also been studied¹¹.

Predation rates of fish were determined by multiplying densities of fish by the mean number of prey consumed per predator¹². Densities of fish collected in 1973 are given in Table 1. Each fish was measured (length and weight), its age and sex determined and its stomach removed. Per cent stomach fullness was also recorded and all prey individuals were enumerated. To convert stomach content data to predation rates, values of stomach evacuation time and diel feeding periodicity were also determined (Fig. 1). While attempts to culture smelt were partially successful, it was not possible to obtain reliable feeding information in the laboratory and all results presented here are derived from field data.

Smelt have a definite seasonal pattern in their predation dynamics. In June, when the hypolimnion is well aerated, the fish consume mostly *Chaoborus* spp. larvae and some immature forms of *Tendipes* spp. and *Pentaneura* spp. near the bottom. There is no evidence to suggest that smelt feed on the bottom of the lake in any season. In July, the fish are found higher in the hypolimnion and their feeding habits reflect their distribution patterns. Approximately 60% of the fish feed on *Daphnia* spp. and the rest consume insect larvae. In August, smelt consume more *Daphnia* spp. than insects. Generally, few copepods are eaten except in August. Burbidge found the same seasonal feeding pattern in 1965–66, except the feeding shift from insect larvae to crustacean zooplankton occurred later in the season¹⁰. His study included the year preceding the occurrence of the first oxygen deficit in the hypolimnion.

The determinations of *in situ* invertebrate predation were obtained using the fluorescence technique which is described in detail elsewhere^{13,14}. Prey are stained with a fluorescent dye (Acridine Orange or Rhodamine B), placed in a grazing chamber and lowered into the lake. The chamber captures 9.8 litres of lake water including a natural assemblage of prey and predators. Simultaneously, the stained prey are released. Predation continues for 1 h, then all animals in the chamber are recovered and scored under a fluorescence microscope. A predator of a stained prey exhibits fluorescent appendages and/or gut tube. Invertebrate predators except *Chaoborus* spp. do not usually consume their prey whole; therefore, it is impossible to ascertain how much of a given prey was consumed, but many partially ingested prey are presumably eliminated from the lake. Mean predation rates, calculated as number of prey partially ingested per predator per time period, were determined in the summers of 1973 and 1974 and densities in 1972, 1973 and 1974.

The major cause of death for the Gull Lake zooplankton community (except insect larvae) is invertebrate predation

Table 1 Smelt densities (animals per m³) for each sampling period, calculated from vertical gill net data

Depth (m)	SS	MT	SR	N
16 June 1973				
1–5	*	*	*	*
6–10	*	2	0.8	*
11–15	0.4	0.4	1.2	*
16–20	*	*	1	*
21–25	0.2	0.6	*	*
26–30	1	0.2	1	0.8
15 July 1973				
1–5	*	*	*	*
6–10	0.2	1.8	0.2	*
11–15	0.8	4.4	0.4	*
16–20	0.8	8.0	2.2	*
21–25	*	2.8	0.8	*
26–30	0.2	*	4.2	*
15 August 1973				
1–5	*	*	0.2	*
6–10	1.4	0.6	0.2	*
11–15	0.2	2.2	0.4	*
16–20	1.4	0.2	*	*
21–25	*	0.2	*	*
26–30	*	*	*	*

SS, sunset; MT, midnight; SR, sunrise; N, noon. *, No fish captured. The gill nets extended the total depth of the central basin. Several mesh sizes were used; however, only the 1.3-cm mesh captured substantial quantities of fish and only these results are reported. The nets were sampled at four time periods during each diel period. In June, the fish were mostly in the 6–15-m range with some individuals near the bottom of the lake. In July, as the surface water warmed, the fish were usually located at 11–20 m with an indication of downward movement at sunrise. In August, there was a notable lack of smelt in the hypolimnion during the period of oxygen depletion. For all dates, few fish were collected at noon. Sonar records have confirmed that fish are absent in the daytime when they probably migrate inshore¹⁰.

Table 2 Comparison of % prey cropped by major vertebrate predator (*Osmerus mordax*) and small invertebrate predator (*Cyclops bicuspidatus*)

Prey	Depth (m)	Density	June		Density	July		Density	August	
			<i>Cyclops</i> (% cropped)	Smelt (% cropped)		<i>Cyclops</i> (% cropped)	Smelt (% cropped)		<i>Cyclops</i> (% cropped)	Smelt (% cropped)
<i>Daphnia</i>	5	5,040	33	<0.1	10,200	22	1	31,100	6	0.3
	20	6,300	69	NC	16,800	8	11	7,250	11	<0.1
<i>Cyclops</i>	5	4,480	1	<0.1	25,200	5	NC	3,080	5	<0.1
	20	2,620	1	NC	15,700	5	<0.1	3,560	5	<0.1
<i>Leptodora</i>	5	10	*	NC	38	*	NC	8	*	63
	20	12	*	NC	38	*	2	14	*	100
<i>Chaoborus</i>	5	11	*	100	25	*	100	13	*	100
	20	14	*	100	7	*	100	15	*	100

Smelt as a population is less effective in cropping zooplankton prey than is *C. bicuspidatus*. Only night values and one invertebrate predator are included to enhance comparability. In addition, only prey species found in smelt stomachs are included. *C. bicuspidatus* consumes several other prey species and has a greater predation impact on the zooplankton community than indicated here¹³. Predation rates for *C. bicuspidatus* were averaged from several experiments in the field and laboratory and then multiplied by *in situ* predator-prey densities to determine number of prey consumed. This value was divided by *in situ* density to estimate % prey cropped, at the appropriate season, depth and time. Although *C. bicuspidatus* cannibalism occurred in all field experiments, there were not enough experiments with this species used as prey, thus, predation rates were difficult to calculate and % prey cropped values are rough estimates. *C. bicuspidatus* does not prey effectively on *Leptodora* and *Chaoborus* and these interactions were treated as not occurring. (*) NC, not consumed in this time-depth period. Densities of zooplankton in the water column were determined from samples by using water bottles or by making horizontal tows with a Clarke-Bumpus zooplankton sampler⁷ and are given as organisms per m³.

(Table 2). *Cyclops bicuspidatus* is an especially effective predator and, given its high densities, can substantially crop the rest of the community. This species possesses several adaptations that contribute to its efficiency¹³. First, the predator does not use vision to locate its prey. It feeds mostly by mechano- or chemo-reception and perhaps selects prey more for their availability than for any other factor. Thus, this predator exhibits an effective density-dependent regulation on the other species. Second, it is highly cannibalistic at high densities, producing short self-damping loops in the ecological network (foodweb) which are stabilising to the ecosystem as well as producing effective regulation of its own population¹³. Third, several members of this species often consume a single prey simultaneously¹³. This group behaviour increases its predation efficiency and also has important ramifications for calculations of nutrient and energy flow. Fourth, *C. bicuspidatus* often nibbles on its prey and does not consume them completely, which makes accurate estimates of energy and nutrient flow difficult. With the fluorescence technique, the percentage of a

prey individual consumed cannot be calculated as only the presence-absence and not amount of fluorescence is quantified¹³.

Smelt consume a broad range of prey species and sizes and do not feed as a stereotyped size-selective fish as is suggested by contemporary dogma¹⁵. Although they may decrease populations of hypolimnetic zooplankton such as *Daphnia pulex* and *Chaoborus* spp., smelt probably have little effect on the myriad of interactions among most zooplankton species^{13,16}. The net effect of smelt on several prey species may be positive as the smelt's high predation rate on insect larvae such as *Chaoborus* spp. (which are voracious zooplankton predators) may indirectly benefit the other prey more than the fish's direct predation harms them. *Chaoborus* spp. populations had decreased in 1973 to 70% of their 1966 densities. These animals are meroplanktonic and they find refuge against smelt predation in the bottom sediments. For Gull Lake, and undoubtedly many other lakes, invertebrate predators constitute the main predation pressure on a wide variety of zooplanktonic prey species. Because there have been so many technical problems in assessing invertebrate predation in the field before the development of the fluorescence technique, many workers have tended to over-emphasise the relative importance of fish predation^{1,3,15}. Fish are definitely more effective predators on a per individual basis but invertebrate predators are disproportionately more abundant than fish. Thus, on a per population basis, invertebrate predators constitute the greatest danger to most zooplankton prey.

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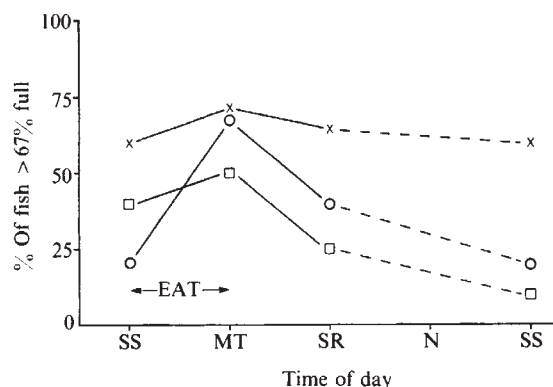


Fig. 1 Feeding periodicity based on stomach fullness shows that most smelt were full at midnight. From sunset to midnight, percentage of fish with stomachs at least 67% full increased markedly and then decreased until dawn to levels similar to those at sunset. Thus, smelt consume most of their prey from sunset to midnight. As fish are relatively empty at sunset and they apparently do not feed in the daytime, most of their feeding is concentrated in a 6-h period after sunset. Hourly stomach evacuation rates were calculated by subtracting the percentage of fish that were at least 67% full at dawn from those 67% full at midnight and dividing this value by six. For all dates (including several not shown) a mean value of 4.3% of the stomach contents was evacuated per hour or in a 6-h period ~25% of the stomach contents were evacuated.

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Autoradiographic localisation of benzodiazepine receptors in the brains of humans and animals

THE benzodiazepine (BZ) drugs are used as anxiolytics, hypnotics, anticonvulsants and muscle relaxants. They are the most widely prescribed of all psychotropic drugs and seem to exert their action by binding to a specific receptor site on brain membranes^{1,2}. This receptor appears to interact with neurotransmission mediated by γ -aminobutyric acid (GABA)^{3–8}. Biochemical studies of BZ receptor distributions in brain reveal large regional variations^{9,10}. An increase in resolution of localisation of BZ receptors would provide greater insight into mechanisms of actions of BZs. In this study, we report for the first time the light microscopic autoradiographic localisation of BZ receptors in mammalian central nervous system (CNS) and, to our knowledge, the first receptor autoradiography in human brain tissue. We observed striking variations in receptor densities in different areas which, in some cases, were species dependent.

The recently developed overall autoradiographic method¹¹ is based on *in vitro* labelling of receptors, since *in vivo* labelling results in low ratios of specific to nonspecific binding^{12,13} and because it is desirable to use human postmortem tissues. Previously, *in vitro* labelling of receptors in brain sections and subsequent coating by nuclear emulsion for autoradiography has been feasible with irreversibly, or nearly irreversibly, binding ligands^{14,15}. However, this approach has been unsatisfactory for most or all diffusible ligands^{11,16}. Our method largely circumvents this problem by apposing a dry layer of emulsion against labelled tissue.

Briefly, brain tissues are placed on brass chucks and frozen in liquid nitrogen. Sections (4–6 μ m) are cut in a cryostat microtome and thaw-mounted onto gelatin-coated slides. The slides are then dipped into 0.17 M Tris-HCl (pH 7.4 at 4 °C) containing 1 nM ³H-flunitrazepam (87 Ci mmol⁻¹, NEN) to label the receptors. Some solutions contain 1 μ M clonazepam to produce sections with nonspecific binding only. The incubations are performed at 4 °C for 40 min at which time the slide-mounted sections are washed for 2 min in buffer alone to reduce nonspecific binding. Routinely, we observed ratios of specific to nonspecific binding of at least 20:1 in preliminary biochemical studies. The binding was saturable with a K_D of 2.5 nM and was blocked only by other BZ drugs. The slides are then rapidly dried (for approximately 30 s) under a stream of cold air and flexible, emulsion (Kodak NTB3 emulsion, 1:1 with H₂O) coated coverslips are attached in the dark by glue to the slide at the end away from the tissue sections. After exposure for 2 weeks at 4 °C, the coverslips are gently bent away from the tissue sections, the latent autoradiograms developed, and the tissues fixed and stained with pyronine Y as previously described¹¹. The assemblies are reapposed and then examined under the microscope.

Male Sprague-Dawley rats (180–250 g) and mice (20–50 g) (Jackson Laboratories) were used in animal studies. Human postmortem tissue samples were obtained at autopsy from a 50-y-old male (3 h post mortem; sudden death with no drug



Fig. 1 Benzodiazepine receptors in human cerebellum. The darkfield photomicrograph in *a* shows the autoradiographic grain distribution over the tissue in *b*. Note the high density of receptors in the molecular layer (M) and in the granule cell layer (G), the reduced density in the Purkinje cell layer (indicated by arrows), and the absence of receptors in the white matter (W). The darkfield photomicrograph in *c* shows the blockade of receptor binding in an adjacent 4 μ m section (brightfield not shown) due to the addition of 1 μ M clonazepam. The conditions for labelling the receptors are outlined in the text. Sections were incubated with 1 nM ³H-flunitrazepam in Tris-HCl (pH 7.4, 0.17 M) for 40 min at 4 °C, and washed for 2 min at 4 °C in the same buffer. The emulsion was exposed for 2 weeks at 2–4 °C. In tissues from two humans, actual grain densities (grains per 1000 μ m² $\pm$ s.d., $n = 6$) for the cerebellar layers were as follows: molecular, 73 ± 9 and 79 ± 3 ; Purkinje cell, 67 ± 8 and 60 ± 18 ; granule cell, 77 ± 3 and 73 ± 8 ; and white matter 13 ± 7 and 8 ± 3 . The corresponding values for rat cerebellum were 84 ± 17 , 53 ± 5 , 35 ± 4 , and 6 ± 1 . Grain densities in the presence of 1 μ M clonazepam were 5–11 grains per 1,000 μ m². The grain densities were counted in at least two preparations in six areas using a calibrated eyepiece grid. Scale bar, 500 μ m.

history) and a 22-y-old male (4 h post mortem; stab wound with no recent drug history) in cooperation with the Medical Examiners Office in Baltimore, Maryland.

In human tissues, we examined the autoradiographic distribution of BZ receptors in cerebellar and cerebral cortical areas including the superior frontal, the precentral and the calcarine areas of the cortex. In all of these areas there was a striking absence of BZ receptors in the subcortical white matter. In the cerebellum, there was a high density of receptors in association with both the granule cell layer and the molecular layer. There was a slight reduction in receptor density in the Purkinje cell layer (Fig. 1; see also Table 1). In the cerebral cortical areas, there were variations in autoradiographic grain densities. The receptor density in the calcarine cortex, for example, was highest in Brodmann¹⁷ layers III, IVA and IVC (Fig. 2, Table 1). This striking variation emphasises the value of this technique in the study of the neuroanatomical aspects of receptor localisation.

The absence of BZ receptors in white matter and the regional variations in receptor density were also observed in brain tissues from rats and mice. In the cerebellum there was a similar high density of BZ receptors in the molecular layer but, compared with the human tissues, there was a markedly reduced level of BZ receptors in the granule cell layer (see Table 1). In the rat and mouse cerebral cortex, there were also variations in the different cortical layers. In the rat, the autoradiographic grain densities in the caudate-putamen and septum were lower than in the cortex and fairly evenly distributed over these structures, although still of a fairly high density. In these same tissue sections we observed an elevation of BZ receptors in and near the islands of Calleja in the ventral forebrain (Table 1).

A striking regional variation in BZ receptors was observed in the rat cervical spinal cord. As in other areas, there were no receptors in the white matter areas, but high levels of receptors were found in laminae II, III, IV, and X while there was a much

lower receptor density in lamina IX which contains the motor neurones. Finally, we observed BZ receptors highly localised to the inner plexiform and ganglion cell layers of the rat retina. The grains were also present to a lesser extent in the nerve fibre and inner nuclear layers (Table 1).

While one cannot rule out some glial localisation of receptors, the striking absence of BZ receptors in white matter and its restriction to grey matter areas are consistent with the notion that the receptors are localised to neurones. The striking variations of receptor densities within grey matter areas suggest that the binding site is not simply a common constituent of all neuronal membranes. Earlier suggestions that the receptors are localised to glial cells¹⁹ are difficult to reconcile with the observed interactions with the neurotransmitter GABA³⁻⁸ and recent reports that these receptors are reduced in *nervous mice*¹⁹⁻²². In addition, we did not observe binding of ³H-flunitrazepam to blood vessels of any size, although the ependymal cell layer around the fourth ventricle showed significant binding of ³H-flunitrazepam which was not displaced by 1 μ M clonazepam and was not apparent with controls for positive chemography.

There have been many biochemical and electrophysiological studies showing an interaction of BZ receptors with GABA receptors³⁻⁸. Our autoradiographic localisations of GABA receptors have not revealed a simple correlation between BZ receptors and GABA receptors labelled with ³H-muscimol in the cerebellum. We did not observe a high density of ³H-flunitrazepam binding sites in and around the Purkinje cells as was observed previously with ³H-muscimol²³. In fact, our initial studies with ³H-muscimol and ³H-GABA in the rat reveal higher receptor levels in the granule cell layer than in the molecular layer (J. M. Palacios *et al.*, in preparation). When GABA (200 μ M) was present during the labelling of the BZ receptor in the rat cerebellum, autoradiographic studies showed that the increases in ³H-flunitrazepam binding occurred in the same proportion in all layers. Our observed high density of BZ receptors in the dorsal horn of the spinal cord does partially overlap with the distribution of glutamic acid decarboxylase (GAD) immunoreactivity in these areas²⁴. These overall results are compatible with the observed interaction between BZ and

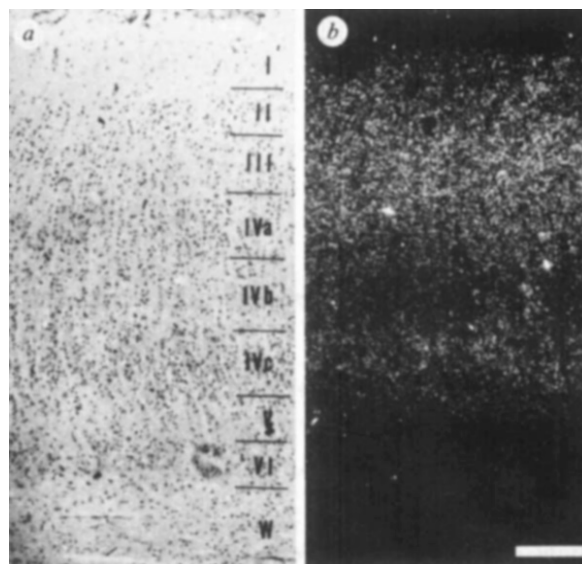


Fig. 2 Benzodiazepine receptors in human calcarine cortex. The darkfield photomicrograph in *b* shows the autoradiographic grain distribution over the tissue in *a* where the cortical layers (I–VI) and the subcortical white matter (W) are labelled. The receptor density varied in the different layers (for example elevated in Brodmann¹⁷ layers III, IVA, and IVC) and was relatively negligible in the white matter. Adjacent sections labelled in the presence of 1 μ M clonazepam showed a loss of the autoradiographic grains with a resultant grain density similar to that shown in *a* and *b* for white matter (not shown). Experimental details are given in the text and in the legend to Fig. 1. Scale bar, 250 μ m.

Table 1 Relative density of benzodiazepine receptors in selected brain regions

Region	Relative grain density
Rat:	
Cerebellum	
Molecular layer	+++
Purkinje cell layer	++
Granule cell layer	++
White matter	0
Striatum	+
Cerebral cortex (frontal)	++ to +++
Corpus callosum	0
Islands of Calleja	+++ to ++++
Spinal cord	
Laminae I, VIII, IX	++ to ++
Laminae II, X	+++
Laminae III, IV, V, VII	++ to +++
Retina	
Inner plexiform layer	+
Ganglion cell layer	+
Human:	
Cerebellum	
Molecular layer	+++
Purkinje cell layer	++
Granule cell layer	+++
Calcarine cortex	
Layers I, II, IVb, V, VI	++
Layers III, IVa, IVc	+++

Tissues were processed and examined as described in text and Fig. 1.

GABA receptors but further suggest that BZ receptors are more widely distributed for additional functions and that only a subpopulation of GABA receptors interact with benzodiazepine receptors.

The wide distribution of BZ receptors through the CNS contrasts with the much more restricted localisations of other receptors, such as the opiate receptors²⁵. The cholinergic muscarinic receptors are similarly widespread but are more localised to telencephalic areas than brainstem areas²⁶. Interestingly, high densities of muscarinic receptors were demonstrated in and around the islands of Calleja²⁶ which is similar to what we have observed in this study.

The species differences in receptor distribution in the cerebellum and possibly in other areas suggest that that extrapolation of results from pharmacological studies with animals to humans must be done with caution. Administration of benzodiazepines has striking clinical effects as mentioned above. While the widespread distribution of BZ receptors in the CNS suggests caution in correlating anatomy and drug action, it is tempting to associate anxiolytic action roughly with receptors in cortical areas²⁷ and muscle relaxant actions with spinal cord receptors.

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Central remyelination restores secure conduction

ONE of the outstanding characteristics of multiple sclerosis and other clinical and experimental diseases associated with demyelination, is the tendency to remit. Although there is good evidence that an important cause of functional loss in such conditions is conduction block produced by the selective destruction of the myelin sheath¹, the mechanisms underlying remission are unknown². It has long been recognised that in certain experimental lesions demyelinated central axons can be remyelinated by the normal myelin-forming cell, the oligodendrocyte³. The new sheaths are, however, abnormally thin and short and it is not known whether they are capable of restoring conduction. A previous investigation was inconclusive⁴. Because of the physiological and clinical importance of this problem, we have now examined conduction serially through the lesion induced by the direct microinjection of lysophosphatidyl choline (LPC) into the cat spinal cord⁵. This lesion has the advantage that the phases of active demyelination and remyelination are well separated, thus permitting a reasonable correlation of the morphological with the physiological changes. We have found that secure conduction is restored with remyelination.

Pairs of platinum or stainless steel stimulating electrodes were permanently implanted at two extra-dural sites over the dorsal columns (usually L1 and L3) in five cats. Recordings were made from the saphenous nerves with stainless steel needles which were accurately repositioned at each session with the aid of tattoo marks. For the recording sessions the cats were anaesthetised with Saffan (Glaxo), paralysed with gallamine triethiodide, intubated and artificially respired to maintain the end-tidal CO₂ level at 4%. Rectal temperature was maintained at 37 ± 0.5 °C. Differential recordings were made with unity-gain cathode followers and Devices pre-amplifiers. The stimulus thresholds were re-determined at each recording session and remained fairly constant over many months. The responses to stimuli at 4 × threshold were averaged ($n = 64$; Biomac 1000) from each nerve after stimulation at each site. Occlusion of the response from the rostral stimulating site as the stimulus strength at the caudal site was increased established that the fibres contributing to the caudally evoked response included those stimulated at the rostral site. The refractory period of transmission⁶ (RPT) was determined from the averaged

response to the second of two stimuli at 4 × threshold at a range of interstimulus intervals⁷. Recordings were made for 1-2 months before and up to 4 months after inducing the lesion. When the responses were stable at successive sessions, injections of 1.5-2.0-μl LPC (0.8%) were made into the dorsal columns. At the end of the experiment the lesions were studied by light and electron microscopy following glutaraldehyde (4%) fixation by perfusion or immersion.

The sequence of morphological events in the LPC lesion has already been reported⁵ and is summarized in Fig. 1. At 3 and 7 days there was extensive demyelination and at these times the amplitudes of the compound action potentials elicited from the rostral stimulating site were markedly reduced in contrast to the control response, which remained unchanged. The conclusion that conduction in many fibres was blocked by demyelination was confirmed by the observation of a large positivity in antidromic recordings made acutely at the lesion site after 7 days.

Remyelination had begun by 14 days, and there was a coincident increase in the number of fibres contributing to the rostrally evoked response (Fig. 1). Over the succeeding weeks the thickness of the new sheaths and the response amplitude increased in step until approximately 81 days, when both stabilised. These later recordings were similar in form to those made before the injection but had a reduced amplitude attributable to the loss of some fibres by Wallerian-type degeneration. There was little change in the latency of the long-term responses. The control response remained constant throughout.

A measure of the security of conduction was obtained from the RPT. In normal fibres, the maximum observed RPT was 1.2 ms. At 3 and 7 days, the range of RPTs of the few fibres still conducting was normal, in keeping with the presence of some histologically unaffected fibres. However, at 14 days, when the compound action potential had increased in size, the majority of fibres had prolonged RPTs (up to 4 ms). Thereafter, the RPT decreased progressively and was normal by 81 days. The RPTs of the control response remained normal throughout.

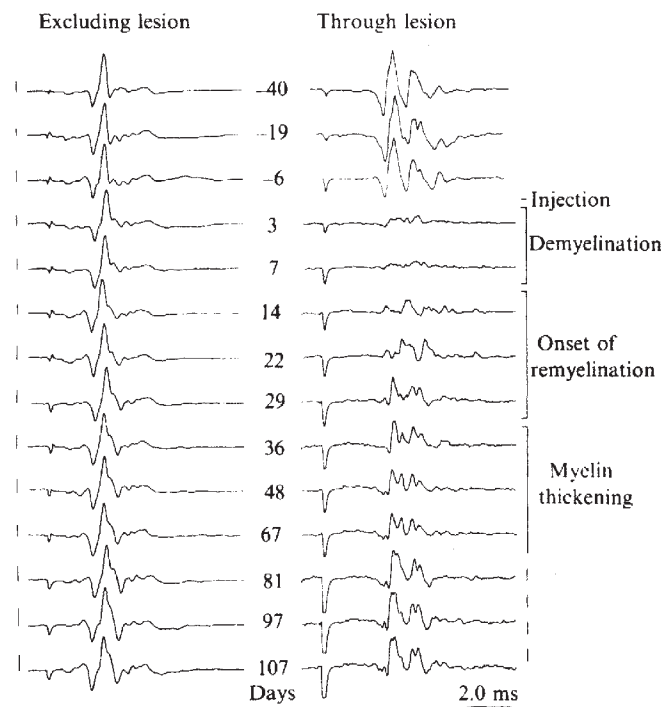


Fig. 1 Saphenous nerve compound action potentials recorded at different times during the development of a focal demyelinating and remyelinating lesion in the dorsal column. To compensate for variables at the recording site the gain on each control response has been adjusted so that their peak-to-peak amplitudes are equal. The corresponding rostrally evoked response has been adjusted by the same factor; each was recorded at 3.3 times the gain of the control.

Vertical calibration bar for each recording, 10 μV.

These experiments have established that conduction begins to return to previously blocked fibres at about the time remyelination begins. Conduction is at first insecure but as the sheaths thicken the RPTs progressively return to normal despite the fact that the internodes remain both thinner and shorter than normal (ref. 5 and W. F. B. and Murray, unpublished observations). The nodal and paranodal morphology is, with few exceptions, normal (W.F.B., unpublished observations). The observation that conduction returns at the same time as new myelin sheaths are formed suggests that it is saltatory in nature. This interpretation is consistent with the return of the RPT to normal. The techniques now available cannot establish whether there is a preceding phase of continuous conduction as in demyelinated⁸ and amyelinated⁹ peripheral axons.

The restoration of conduction by remyelination is probably of only limited importance to the remissions and delayed evoked potentials¹⁰ seen in multiple sclerosis, as remyelination is limited in this disease¹¹. However, it will probably be more important in mediating the recovery seen after demyelination induced by certain compressive lesions of the spinal cord which, at least experimentally, are associated with the formation of new myelin sheaths with similar characteristics to those found in the LPC lesions^{12,13}. Although the reason for the striking differences in the extent of remyelination between different types of lesion is unknown¹⁴, the observation of restoration of secure conduction by remyelination suggests that further investigation of techniques for promoting central remyelination would be well worthwhile¹⁵.

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The optical mechanism of the eye of *Limulus*

THE compound eyes of many marine and some terrestrial arthropods have smooth surfaces with no convex curvature to the facets. This means that image formation by ordinary spherical refraction is impossible. Nevertheless, apposition eyes of this kind form images behind each surface facet^{1,2} (Fig. 1b) and the question has repeatedly arisen as to how these images are produced. Exner chose the king crab, *Limulus*, as providing the definitive example of such an eye, and from a mixture of observation and theory came up with the idea of a lens-cylinder,

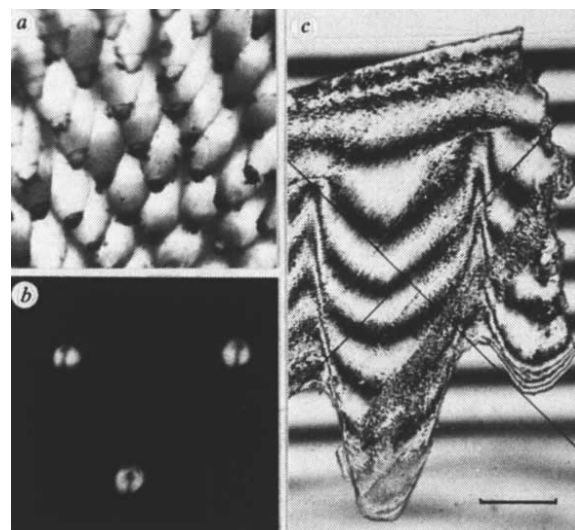


Fig. 1 *a*, Inner surface of the cornea of *Limulus polyphemus* showing the cleaned tips of the crystalline cones. *b*, Images photographed at the cone tips. The object was a black arrowhead on a light circular background whose diameter subtended 8.9° at the eye. The preparation was immersed in seawater. Average separation of the cone tips is 255 μm. *c*, Appearance of a thin parallel-sided frozen section through the centre of a cone using shearing interference optics and a fringe-field eyepiece (Vickers). The whole fringe pattern is visible on the right, with the zero-order (black) fringe at the top. This fringe reappears in the specimen under the crosswires, and it can be seen that in the cone centre it is displaced by just over 4 wavelengths ($\lambda = 542$ nm) and that away from the cone axis this displacement decreases roughly parabolically to about 2 wavelengths. The refractive index (n) at any point can be found from the path difference (PD):

$$PD = m\lambda = t(n - 1.334), \text{ or } n = m\lambda/t + 1.334$$

where m is the number of wavelengths by which the appropriate fringe is displaced and t is the section thickness (13.0 μm). The tip of the cone overlaps the microscope's second image, and the last fringe is lost.

a flat-ended optical device in which the refractive index decreases from the central axis to the periphery in a roughly parabolic manner¹. This arrangement will form an image (Fig. 2a), and devices of this kind have actually been manufactured^{3,4}. Exner's theory has long been considered to be the correct account of image formation in this type of eye⁵. However, Levi-Setti, Park and Winston² have recently produced a fundamentally different explanation of image formation in *Limulus* eyes (Fig. 2b). This was based on the idea that each optical element, or crystalline cone, concentrated light by reflection, not refraction, and that it was the shape of the crystalline cone's reflective surface, and not its internal refractive index gradient, that led to the image-forming properties of the structure. This principle had already been used in another optical device, the 'ideal light collector' (ref. 6) which was invented as a tool for concentrating faint radiation. In this report I discuss the two suggested optical mechanisms for the eye of *Limulus*, and present evidence from interference microscopy that Exner's theory is probably the correct one.

One difference between the refracting (Fig. 2a) and reflecting (Fig. 2b) explanations is that the former predicts an inverted image and the latter an erect one. (It is, in fact, a design feature of the ideal light collector that the parabolic profile on the left focuses light onto the lower tip of the right profile, thereby excluding light making larger angles with the axis than that shown in the diagram⁶.) The images seen at the tips of the cones in *Limulus*, are, in fact, inverted (Fig. 1b).

A second difference is that in the refracting mechanism there should be a relatively gradual refractive index change from centre to periphery of each cone, whereas in the reflecting

mechanism this should be abrupt, so that total internal reflection can occur at the interface. The exact form of the gradient in a refracting lens-cylinder was not known to Exner¹, but was worked out later by Fletcher, Murphy and Young⁷. Their solution, for a structure of length F and central refractive index n_0 is

$$n = n_0 \operatorname{sech}(\pi r/2F) \quad (1)$$

where n is the refractive index at a radial distance r from the axis of the cone. Some of these curves, for different values of F , are plotted in Fig. 3a.

It is possible to determine which prediction is correct by interference microscopy. An interference microscope splits light from a single source into two beams, one of which passes through the specimen and the other through the bathing medium. These beams are recombined in the objective, and where the optical path-length through the specimen differs from that in the medium, interference occurs, resulting in colours in white light, or distinct dark and light fringes in monochromatic light. In the microscope I used, the interference version of a Vickers M41, there are two distinct ways of viewing the specimen. It can be seen against a uniform background with a constant path difference across the field of view, in which case optical inhomogeneities within the specimen show up as a series of fringes representing 'contour lines' of refractive index, provided the specimen is of uniform thickness. Alternatively, a fringe-field eyepiece incorporating a quartz wedge can be used; this produces a series of visible fringes in the field of view, and the effect of a specimen is to displace these in such a way that the refractive index profile through the section is depicted directly as the pattern of fringe distortion. The first method is capable of giving accurate local measurements of refractive index, but the latter is much more useful for demonstrating the shape of the whole refractive index profile. From their studies Levi-Setti *et al.*² and Carricaburu⁸ concluded that the cone itself had a high and fairly uniform refractive index, but was surrounded by a lower refractive index, less homogeneous matrix, and that the transition from cone to matrix was abrupt enough for total internal reflection to occur.

Figure 1c shows otherwise. This shows a frozen section, 13.0 μm thick (the exact thickness was determined from path difference measurements in two media of different refractive index, water and air in this case) taken along the axis of a cone from an excised eye, kept in seawater containing 1% formaldehyde as a preservative. The eye used for sectioning retained the same image-forming properties as freshly excised material, and so was presumably optically identical. The photograph shows a series of fringes that have a roughly parabolic appearance, curving only gently in the centre of the cone, but increasingly more steeply into a trough that represents the centre of the inter-cone matrix. There is no sudden boundary, and when the fringe pattern is translated into a refractive index profile, this is found to coincide very closely with that of an Exner lens cylinder^{1,7} 600 μm long (the actual length of the inhomogeneous

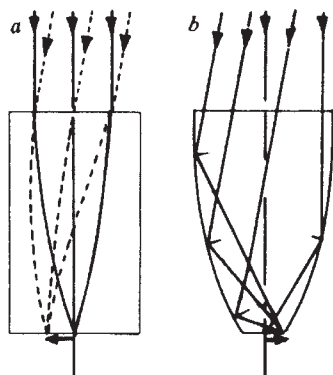


Fig. 2 Optical mechanism of *Limulus* crystalline cones. a, The refracting mechanism of Exner¹; b, total internal reflection mechanism of Levi-Setti, Park and Winston². Scale bar, 100 μm .

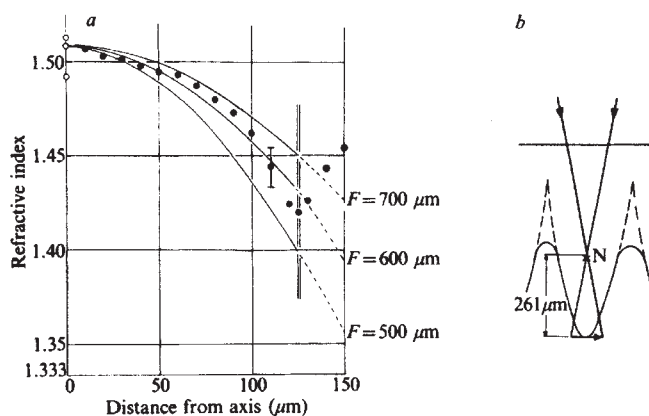


Fig. 3 a, Refractive index gradient from centre to periphery of a single cone. The filled circles are means of six measurements made on each side of the three principal fringes in Fig. 1c. The range of the measurements is shown for the most variable point by the vertical bar. Open circles on the ordinate are central refractive indices of three different cones. The continuous curves are three solutions of equation (1), with $n_0 = 1.51$, and three values for F . Excluding the outer cornea, 100 μm thick, which does not distort the fringes and is optically homogeneous, the axial length of the 'lens-cylinder' part of the cone (F) is about 580 μm . The theoretical curves (dashes) continue to fall indefinitely, but the points begin to rise again at the double vertical line as one cone ends and the next begins. b, The location of the nodal point (N) in a cone, based on image magnification (Fig. 1b), is indicated by the intersection of image-forming rays. The small cross indicates its position as predicted from equation (2).

portion of the cone is 580 μm) with a central refractive index of 1.51 (Fig. 3a). Note that equation (1) contains no arbitrary constants; the fit of theory and data thus means that the profile shown in Figs 1c and 3a must give an image at the cone tip.

By way of confirmation, the focal length of the cone (f , defined as image size/angle in radians subtended by an object at infinity) also coincides with that predicted by Fletcher *et al.*⁷ They give

$$f = 2F/\pi n_0 \quad (2)$$

which for $F = 600 \mu\text{m}$ and $n_0 = 1.51$ gives a value for f of 253 μm . The measured value, obtained directly from the images shown in Fig. 1b, was 261 μm . The receptor clusters, situated just beneath the cone tip, have a diameter of about 35 μm , so that for $f = 261 \mu\text{m}$ each cluster should subtend an angle of 7.7° , which is almost identical to the inter-ommatidial angle in the centre of the eye.

These three observations, the inverted image, the existence of the refractive index profile required for a refracting lens-cylinder and the finding that the focal length is just what this model would predict, must be taken to mean that Exner's enlightened guess was quite correct (Fig. 2a) and that the reflection model (Fig. 2b) is not the way in which the images are produced.

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GTP hydrolysis in intact rod outer segments and the transmitter cycle in visual excitation

THE light-sensitive dark current of vertebrate retinal rods is the main metabolic load on the receptor cells, turning over the cytoplasmic Na^+ every 1–6 min (refs 1–4) and the cytoplasmic ATP as often as every 20 s (refs 5, 6). However, a second process in the rod outer segment (ROS), the transmitter cycle, probably demands metabolic free energy during visual excitation. Photons absorbed in the intracellular rod disks reduce the dark current traversing the nearby plasma membrane by altering the cytoplasmic level of a diffusible intracellular transmitter substance^{7–10} that may be free Ca^{2+} (refs 11–14). As tens to thousands of transmitter particles must be released and removed in a single photon response^{15–17}, the transmitter cycle should cause fast metabolic events in ROSs even when they are isolated from those parts of the receptor cells that sustain the dark current. We report here that light causes ROSs to destroy GTP and form GDP at a rate sufficient to meet the predicted requirement of the transmitter cycle. Briefly, ROSs with intact plasma membranes and normal cytoplasmic nucleotide content were isolated within a few seconds from live frog retinas; aliquots of the ROS suspension were exposed to flashes of light, and the cytoplasmic levels of ROS nucleotides monitored as a function of time. The experimental procedure permits changes in cytoplasmic nucleotides in ROS to be distinguished from those involving nucleotides in the suspending medium or bound to rod disk membranes that are not enclosed within an intact plasma membrane.

Rana pipiens or *Rana catesbiana* were dark adapted for 4 h and their retinas removed by dissection in oxygenated Ringer solution containing 10 mM glucose. All operations were done under IR illumination with the aid of image converters. Four retinas were placed in a plastic syringe barrel of internal diameter 1.44 cm in 1.5 ml of frog Ringer solution containing 0.9 mM Ca^{2+} , 10 mM glucose and 0.5 mM phosphate. Potato apyrase (Sigma), 1 U ml⁻¹, was added to split extra cytoplasmic nucleoside di- and triphosphates to monophosphates. pH was buffered to 7.1 with PIPES and the retinas were slowly tumbled with a stream of O_2 bubbles. After 3 min, a cylindrical Kel-F spindle 0.89 cm in diameter was dropped axially into the syringe barrel. The spindle turned at 3,900 r.p.m., agitating the retinas in a hydrodynamic shear for 10 s. The spindle was withdrawn and the contents of the syringe expelled with a rubber piston through a coarse platinum mesh and a nylon mesh with 37- μm holes on a side. The filtrate contained ROSs, rod cell nuclei and a few erythrocytes (Fig. 1a). When a sample of the suspension was made 10 μM in didansyl cystine (DDC) and examined under 365 nm excitation, many non-fluorescent outer segments with intact plasma membranes were seen (Fig. 1b)¹⁸. When such suspensions were fractionated on metrizamide density gradients, the DDC-staining ROS held less than 10% as much acid-extractable nucleotides per unit particle volume as did the unstained ROS (W. E. R. and W. A. H. in preparation). As 20–35% of the ROS in the suspensions were DDC-excluding the contribution of nucleotides bound to DDC-staining rods to our measurements could usually be neglected.

ROSs were separated from their suspending medium by centrifugal filtration through silicone oil¹⁹. Aliquots (100- μl) of the rod suspension were layered into 1.5 ml Eppendorf centrifuge tubes previously loaded with 0.1 ml of 0.6 M HClO_4 brought to density 1.06 with glucose and overlaid with 0.4 ml of a mixture of Dow-Corning types 555 and 200 silicone fluids of density 1.04 at 25 °C. At selected times a loaded tube was placed in an Eppendorf centrifuge and its contents exposed to a 2-ms flash of 520–590 nm light which reached the rod suspension through the translucent polyethylene tube cap. After a timed

delay the centrifuge was run for 10 s at 16,000g. The ROSs and other particles in the upper aqueous layer passed into the lower HClO_4 layer and became packed as a button. Control experiments with ROSs suspended in Ringer solution containing tritiated water showed that the total volume transferred between the aqueous layers was about three times that of the particles. Particle transfer between the upper and lower aqueous layers was complete within less than 4 s of the start of centrifugation. The lower aqueous layers containing soluble constituents of the cellular particles were neutralised with KOH and



Fig. 1 Photomicrographs of bullfrog ROS suspensions prepared as described. Many rod cell nuclei (n) and a few erythrocytes (e) are present. The nucleoside phosphates are more than 10 times as abundant in ROSs that do not stain with DDC than in those that do. a, Dark field, 546 nm illumination; b, DDC fluorescence, excited at 365 nm and observed at wavelengths greater than 450 nm (see ref. 18).

K_3PO_4 and analysed by high-pressure liquid chromatography on Partisil 10-SAX (Whatman) with a 40-min gradient of KH_2PO_4 , pH 3.5, running from 0.05 M to 0.75 M. Nucleotide yields were expressed as mol per l of non-DDC-staining rod water. The detection limit for A and G nucleotides was about 60 μM .

The only nucleotides detected in the extracts were mono-, di-, and triphosphates of adenosine and guanosine, and a trace of UTP. Figure 2a shows the adenine and guanine nucleotides of a suspension that was sampled over a 5-min period in darkness after collection from the live retinas. There was a slow loss of all forms of A and G that could be shown in control experiments to

be due to leakage from ROSs into the suspending medium. Ouabain (50 μ M) added to the ROS suspension had no detectable effect on the loss of ATP or ADP in darkness: no ATPase activity seemed directed by ROSs towards their own internal ATP. Guanosine phosphates were usually more abundant than those of adenosine. The total nucleoside phosphate content of ROS cytoplasm (including bound forms released during the 5–10-min extraction period in HClO_4) exceeded 5 mM, as previously reported⁵. The triphosphate:diphosphate ratios of the adenine and guanine systems differed widely, that of the G system being much lower even with stringent precautions against exposure of the ROSs to light. The ATP:ADP ratio was very sensitive to the oxygenation of the retinas before separation of the ROSs, dropping from 4–7 to about 1 if the O_2 bubbles were stopped for 1 min before the ROSs were sheared from the retinas.

When ROSs were illuminated with flashes that bleached 1% of their photopigment, there was little effect on the levels of ATP or ADP (Fig. 2*b*). The extractable GDP fell within 4 s by 1.0 ± 0.1 mM ($n = 7$). Then about two-thirds of the extractable GTP disappeared in 20 s and an equal amount of GDP was formed. After 30 s there was a small increase in GMP in some experiments. After allowing for leakage of nucleotides from the ROS, there was little light-induced conversion of ATP into ADP or AMP in the first 20 s after the bleaching light flash. The light-induced changes in nucleotide levels were unaffected by lowering the free Ca^{2+} activity to 1 μ M or

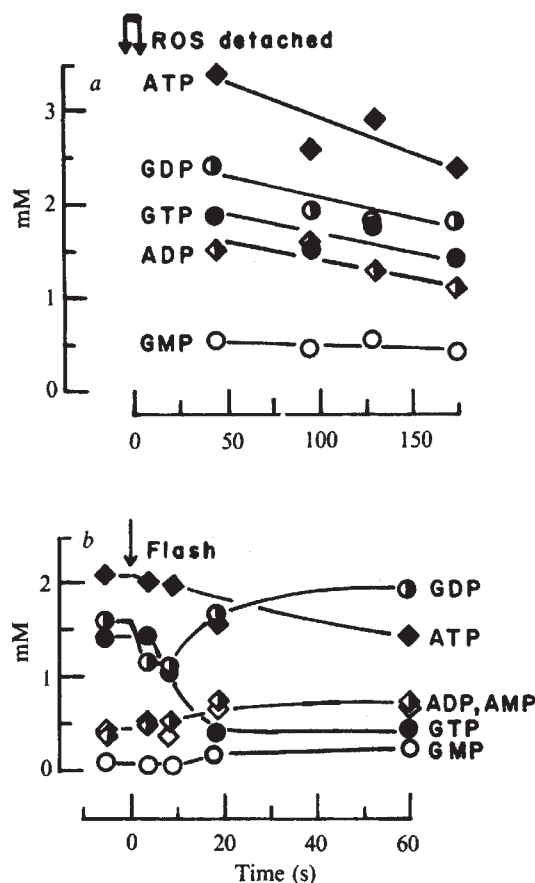


Fig. 2 Nucleoside phosphate levels in intact isolated frog ROSs: *a*, in darkness with time scale beginning the instant that separation from retinas began; *b*, after a 2-ms flash of green light that bleached 1% of the rhodopsin chromophores, time scale beginning at the flash. Light had little effect on ATP or ADP levels but produced a very fast disappearance of GDP followed by a conversion of GTP to GDP with a half time of about 10 s. The values have been corrected for the small slow loss of all nucleotides that occurs in the dark during the 5–10-min course of the experiments.

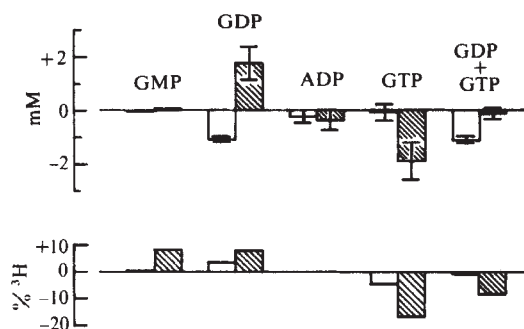
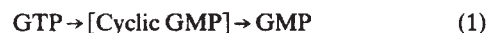


Fig. 3 Changes in guanine nucleotide levels in frog ROSs following a 2-ms 560-nm light flash that bleached 1% of the rhodopsin. Data from four experiments similar to that of Fig. 2*b*. Aliquots of ROS suspension were extracted after centrifugal filtration through silicone oil (see text) before (*a*), 4 s (*b*) and 64 s (*c*) after the bleaching flash. The observed nucleotide level in each aliquot was first corrected for leakage (Fig. 2*a*) by normalising all values to those that would have been obtained if the cytoplasmic ATP + ADP were exactly 5 mM. Open bars, change in nucleotides 4 s after flash. Hatched bars, change in nucleotides between 4 s and 64 s after light flash. Upper row, total extractable nucleotides. Mean of four experiments. Error bars, s.e.m.; lower row, ^3H -labelled nucleotides from ROS of two retinas incubated for 1 h in labelled guanosine in stirred, oxygenated glucose Ringer solution. At 4 s total extractable GDP dropped by 1 mM (process (3)), and both total and ^3H -labelled GTP dropped slightly between 4 s and 64 s. Total GTP was quantitatively replaced by GDP, while the decline in ^3H -labelled GTP was accompanied by the appearance of roughly equal amounts of GDP and GMP. There was no significant change in ATP in the 60 s following the flash.

adding 1 mM of the cyclic nucleotide phosphodiesterase inhibitor, isobutylmethylxanthine, to the ROS suspension.

Do all the guanine nucleotides occur in one light-sensitive pool? The experiments summarised in Fig. 3 suggest that they do not. The upper line of bars in the figure shows net changes in the total extractable guanine nucleotides in four experiments similar to that of Fig. 2*b*. In the first 4 s after the flash, the usual large drop in GDP was seen. Between 4 s and 64 s, GTP dropped and GDP rose by similar amounts. There was no significant change in the GMP level. The lower row of bars denotes changes in the acid-extractable tritiated nucleotides from ROSs of two retinas that had been incubated for 1 h in darkness in stirred, oxygenated glucose Ringer solution containing $20 \mu\text{Ci ml}^{-1}$ of $[8\text{-}^3\text{H}]\text{guanosine}$ (NEN). The specific activity of GTP recovered from the ROSs was about 1% of that of the guanosine. The GTP:GDP ratio for the labelled nucleotides was 3.8, whereas that for the total extractable GTP and GDP was 0.96. The ATP:ADP ratio was 7.6. Thus, the exchangeable guanine nucleotides had a phosphate much closer to that of the adenine nucleotides. This would be the case if about 74% of the extractable GDP in the ROS were bound to inexchangeable sites. The flash caused a small increase in $[8\text{-}^3\text{H}]\text{GDP}$ at 4 s matched by an equal loss of tritiated GTP.

Control experiments on quick-frozen frog retinas showed that the labelled nucleotides were in the layer of outer segments and sedimented with intact ROSs. Thus, the guanine nucleotide pool of frog ROSs must consist of at least two parts: an exchangeable part comprising as much as 25% of the total, apparently following two parallel light-induced pathways:



and an inexchangeable part that shows process (2) and an additional reaction:



Process (1) is to be expected, as rod disks contain both guanylate cyclase²³⁻²⁵ and cyclic nucleotide phosphodiesterase²⁶⁻²⁹. Because the main product of GTP metabolism in illuminated ROSs is GDP, not GMP, either process (2) is the main biochemical pathway for destruction of GTP, or GMP formed by process (1) must be rapidly phosphorylated to GDP.

Process (3) is a surprise. It is hard to reconcile with the protein inventory in the ROS. The binding sites are not likely to be on rhodopsin molecules because only one GDP is bound for every 2-2.5 rhodopsins and because in ROSs whose plasma membranes exclude DDC, at least 90% of the GDP will leak out on hydrodynamic shearing that renders the ROSs leaky to DDC. Thus, the binding sites must be able to leak out of the outer segments along with their bound GDP. The GDP binding resembles that shown by tubulin³⁰. We have found tubulin in DDC-negative ROSs by SDS gel electrophoresis, but the abundance of tubulin monomers is less than 1 per 10 rhodopsin chromophores (W.E.R. and W.A.H., unpublished). The light-induced binding reaction shows a large chemical gain: bleaching of 1 rhodopsin in 100 causes 1 GDP to become inextractable for every 2 photopigment chromophores in the ROS.

The light-induced conversion of GTP to GDP is much faster than that observed in isolated ROS disk membranes^{20-22,31}. In intact ROSs at least 100 GTP molecules are converted to GDP for each photon absorbed by photopigment molecules during the 20 s following a flash, whereas less than 30 GTP molecules are split by ROS disks in a similar time for each photoisomerised rhodopsin chromophore^{20,21}. As only 1 rhodopsin in 1,000 must be photoisomerised to activate the disk GTPase fully, the GTPase activity (if that is what process (2) represents) in the intact ROS must be more than 20 times that previously seen. It would be sufficient to drive a mechanism that removes 1,000 transmitter particles previously released in a photon response¹⁵.

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Potential of Ca^{2+} -dependent K^+ activation by theophylline is independent of cyclic nucleotide elevation

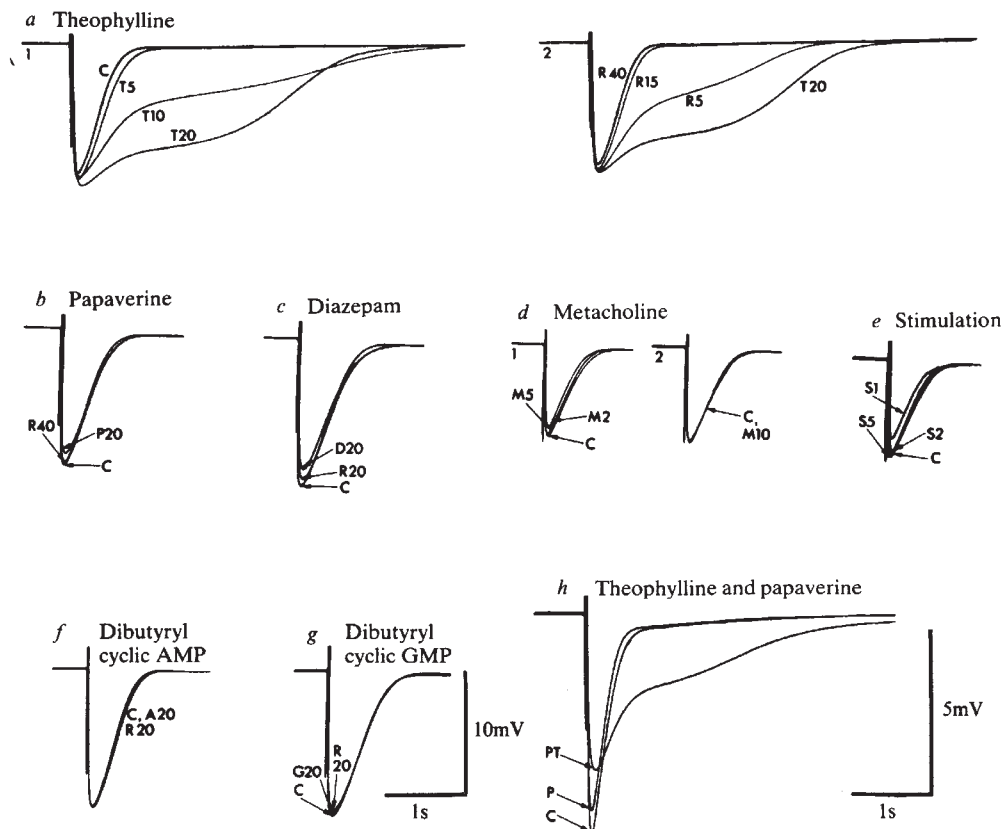
THE action potential in many nerve cells is followed by an after-hyperpolarisation (AH). In several types of neurone, this AH consists of two components: a brief voltage-sensitive K^+ conductance, and a longer Ca^{2+} -sensitive K^+ conductance (refs 1-5, see ref. 6 for review). In sympathetic neurones of the bullfrog, theophylline potentiates this Ca^{2+} -sensitive K^+ conductance⁴. As theophylline is a well known inhibitor of phosphodiesterase⁷, the enzyme which catabolises cyclic nucleotides, we examined the role of cyclic nucleotide metabolism in the regulation of the spike AH. We used both electrophysiological and biochemical methods to investigate whether potentiation of the spike AH by theophylline is correlated with cyclic nucleotide elevation in bullfrog sympathetic neurones. Our results indicate that the potentiation of this Ca^{2+} -dependent K^+ conductance is apparently independent of cyclic AMP and cyclic GMP levels, and suggest that this potentiation by theophylline is not due to inhibition of phosphodiesterase.

The spike AH and other membrane potentials generated in sympathetic neurones were recorded by the sucrose gap technique, as reported previously⁴. Figure 1 shows the spike AH recorded following procedures which would be expected to increase cyclic nucleotide concentration. Theophylline (5 mM) increased the duration of the spike AH (Fig. 1a). Two other phosphodiesterase inhibitors, papaverine (0.1 mM)⁸⁻¹⁰ and diazepam (0.1 mM)¹¹⁻¹³, however, had little effect on the duration of the spike AH (Fig. 1b, 1c). Stimulation of the cholinergic preganglionic nerve fibres which synaptically innervate the neurones in these sympathetic ganglia increases the content of both cyclic GMP and cyclic AMP^{13,14}. However, neither the administration of the cholinergic agonist methacholine nor preganglionic stimulation prolonged the duration of the spike AH (Fig. 1d, 1e).

Figure 1f and g shows that the application of dibutyryl cyclic AMP (3 mM) or dibutyryl cyclic GMP (3 mM) also did not significantly alter the spike AH. As shown in Fig. 1h, theophylline (5 mM) increased the duration of the AH in the presence of papaverine (0.1 mM). This observation raised the question of whether the increase in AH duration by theophylline is due to inhibition of phosphodiesterase, as prior inhibition of this enzyme by papaverine might prevent any physiological action of theophylline that is mediated by phosphodiesterase inhibition. To evaluate this question, and to confirm that the experiments shown in Fig. 1 did alter cyclic nucleotide content, we measured both cyclic AMP and cyclic GMP concentrations by radioimmunoassay¹⁵.

Figure 2 shows the change in cyclic AMP and cyclic GMP concentrations which were produced by the various experimental procedures shown in Fig. 1. The content of both cyclic AMP (Fig. 2a) and cyclic GMP (Fig. 2b) was elevated by the administration of methacholine and by each of the three phosphodiesterase inhibitors, theophylline, papaverine and diazepam. The interaction of papaverine and theophylline on cyclic nucleotide content is shown in Fig. 2c and d. If the potentiation of the spike AH induced by theophylline in the presence of papaverine results from further inhibition of phosphodiesterase, one would expect these two substances to have an additive effect on cyclic nucleotide levels. However, no additive effect was observed (Fig. 2c, d). In the presence of papaverine, theophylline reduced the concentrations of both cyclic AMP (Fig. 2c) and cyclic GMP (Fig. 2d) towards the control concentration seen before papaverine was added. Although the reason for this theophylline-induced decrease in cyclic nucleotide content is

Fig. 1 Action potential after-hyperpolarisation (AH) recorded in conditions expected to increase cyclic nucleotide concentration. *a1*, Effect of theophylline on spike AH. Control AH before theophylline added (C), and superimposed records of spike AH after superfusion with Ringer's solution containing 5 mM theophylline for 5 min (T5), 10 min (T10) and 20 min (T20). Note prolongation of AH duration. *a2*, Recovery from theophylline effect. Spike AH after 20 min in 5 mM theophylline (T20), and superimposed recovery records of AH recorded 5 min (R5), 15 min (R15) and 40 min (R40) after beginning theophylline washout. *b*, Effect of papaverine on spike AH. Control spike AH before papaverine added (C), and superimposed records of AH after 20 min superfusion with 0.1 mM papaverine (P20), and recovery recorded 40 min after beginning papaverine washout (R40). *c*, Effect of diazepam on spike AH. Control AH before diazepam (C), and superimposed records of AH recorded after superfusion with 0.1 mM diazepam for 20 min (D20), and recovery recorded 20 min after beginning diazepam washout (R20). *d*, Effect of methacholine on spike AH; in the presence of 70 μ M (-)tubocurarine, *d1*, Control AH before methacholine (C), and superimposed records of AH recorded after superfusion with 1 mM methacholine for 1 min; records taken 2 min (M2) and 5 min (M5) after methacholine administration. *d2*, Control record (C) from same experiment as *d1* with superimposed record 10 min after administration of 1 mM methacholine for 1 min (M10). *e*, Effect of synaptic stimulation on spike AH. Control AH before stimulation (C), and superimposed records of AH recorded 1 min (S1), 2 min (S2) and 5 min (S5) after preganglionic stimulation. The sympathetic chain was stimulated between the eighth and ninth ganglia supramaximally with 1-ms pulses for 2 min at 10 Hz (see ref. 14). *f*, Effect of dibutyl cyclic AMP on spike AH. Control AH before dibutyl cyclic AMP (C), and superimposed records of AH recorded after superfusion with 3 mM dibutyl cyclic AMP for 20 min (A20) and recovery record 20 min after beginning dibutyl cyclic AMP washout (R20). *g*, Effect of dibutyl cyclic GMP on spike AH. Control AH before dibutyl cyclic GMP (C), and superimposed records of AH recorded after superfusion with 3 mM dibutyl cyclic GMP for 20 min (G20) and recovery record 20 min after beginning dibutyl cyclic GMP washout (R20). *h*, Effect of theophylline on spike AH in the presence of papaverine. Control AH before addition of either drug (C), and superimposed record of AH recorded after superfusion with 0.1 mM papaverine (P). The third superimposed trace was recorded 20 min after the addition of 5 mM theophylline to the Ringer's solution containing papaverine (PT). Note the increased duration of the spike AH after the addition of theophylline. The experiments were carried out on the ninth or tenth paravertebral sympathetic ganglion of the bullfrog by the sucrose gap method. Action potentials were elicited by antidromic stimulation once per minute, as reported previously⁴. The depolarising phase of the action potential was obscured by the stimulus artefact. The spikes were truncated for the purpose of illustration. All records illustrated were obtained using a Brush 280 pen recorder. The composition of the Ringer's solution was (mM): NaCl 100; KCl 2; CaCl₂ 1.8; Tris-HCl 16 (pH 7.2); glucose 1 g l⁻¹.



not clear, it may have important implications for our understanding of cyclic nucleotide metabolism.

Our data show that although theophylline, papaverine, diazepam, methacholine and preganglionic stimulation increase the concentrations of both cyclic AMP and cyclic GMP, only theophylline prolongs the duration of the spike AH. This indicates that the duration of the spike AH does not seem to be associated with the elevation of cyclic AMP and cyclic GMP. Consistent with this is the observation that the administration of dibutyl cyclic AMP or dibutyl cyclic GMP did not significantly affect the spike AH. The observation that theophylline reduced cyclic nucleotide content in the presence of papaverine but potentiated the spike AH, indicates that the potentiation of this Ca²⁺-dependent K⁺ conductance is independent of cyclic nucleotide elevation. This observation also suggests that the potentiation of the AH by theophylline is not due to phosphodiesterase inhibition. This interpretation has additional significance in view of the fact that the potentiation of various responses in neurones by theophylline has often been cited as evidence for cyclic nucleotide mediation of such phenomena¹⁶⁻¹⁸.

In the sympathetic neurones studied here, potentiation of the spike AH by theophylline is dependent on the presence of extracellular Ca²⁺ ions⁴. During an action potential, in addition

to an influx of Na⁺ ions there is an influx of Ca²⁺ ions into these neurones¹⁹. It is likely that this Ca²⁺ influx activates the K⁺ conductance which prolongs the duration of the spike AH. Busis and F.F.W.⁴ suggested that theophylline may potentiate the spike AH by increasing the stimulus-induced entry of Ca²⁺ ions. Recently, Klein and Kandel²⁰ reported that phosphodiesterase inhibitors prolong the duration of a Ca²⁺ spike in a sensory neurone of *Aplysia*. They showed that the prolongation of this Ca²⁺ conductance could also be produced by synaptic stimulation and suggested that cyclic AMP was mediating this effect of the neurotransmitter, serotonin. Cyclic nucleotides have also been reported to prolong a Ca²⁺-dependent K⁺ activation in spinal motoneurons²¹. Our data, on the other hand, indicate that the prolongation of the Ca²⁺-dependent K⁺ activation in sympathetic neurones is independent of cyclic nucleotide elevation. It thus seems that various mechanisms may be regulating Ca²⁺-dependent conductance changes in different types of neurone. Such a diversity is further illustrated by the recent observation that Ca²⁺-dependent conductance changes can be reduced rather than enhanced by neurotransmitter action^{22,23}.

The AH following an action potential produces the absolute and relative refractory periods which prevent spike reactivation after short time intervals and thus limit spike firing frequency. An understanding of the cellular mechanisms controlling the

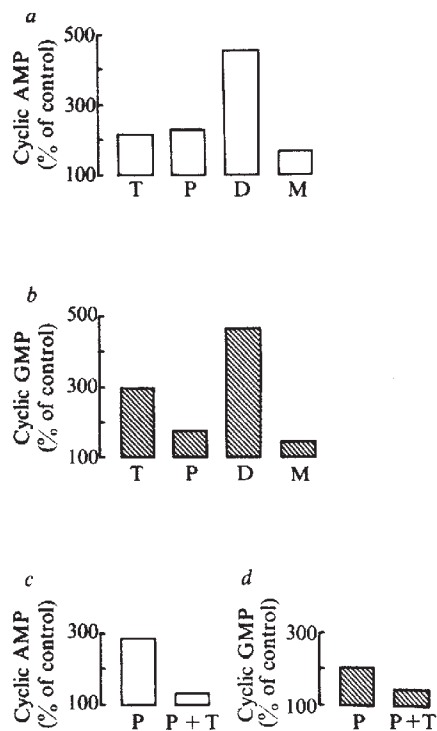


Fig. 2 Effect of phosphodiesterase inhibitors and methacholine on cyclic nucleotide concentration in bullfrog sympathetic ganglia. *a*, Increase in cyclic AMP content produced by theophylline (T), papaverine (P), diazepam (D) and methacholine (M) illustrated as per cent of control. For each experiment, $n = 5$ or more. The changes in cyclic AMP concentrations were as follows, expressed as pmol per mg protein (mean $\pm$ s.e.m.). Incubation of ganglia with 5 mM theophylline for 20 min increased cyclic AMP concentration from 10.53 ± 1.63 to 23.26 ± 2.42 ($P < 0.005$). Incubation with 0.1 mM papaverine for 20 min increased cyclic AMP from 17.83 ± 1.61 to 41.9 ± 1.37 ($P < 0.001$). Incubation with 0.1 mM diazepam for 20 min increased cyclic AMP from 20.5 ± 2.9 to 93.17 ± 32.3 ($P < 0.05$). Incubation with 1 mM methacholine for 2 min increased cyclic AMP from 40.05 ± 6.69 to 70.08 ± 12.21 ($P < 0.1$) determined 5 min after M administration, in the presence of $70 \mu\text{M}$ (-)-tubocurarine. *b*, Increase in cyclic GMP content produced by theophylline, papaverine, diazepam and methacholine illustrated as per cent of control. For each experiment, $n = 5$ or more. The changes in cyclic GMP concentrations were as follows, expressed as pmol per mg protein (mean $\pm$ s.e.m.). Incubation of ganglia with 5 mM theophylline for 20 min increased cyclic GMP concentration from 0.97 ± 0.12 to 2.92 ± 0.4 ($P < 0.001$). Incubation with 0.1 mM papaverine for 20 min increased cyclic GMP from 0.98 ± 0.07 to 1.78 ± 0.18 ($P < 0.005$). Incubation with 0.1 mM diazepam for 20 min increased cyclic GMP from 0.29 ± 0.03 to 1.38 ± 0.29 ($P < 0.005$). Incubation with 1 mM methacholine for 2 min increased cyclic GMP from 0.58 ± 0.13 to 0.84 ± 0.12 ($P < 0.4$) determined 5 min after M administration, in the presence of $70 \mu\text{M}$ (-)-tubocurarine. *c*, Effect of theophylline on the % increase in cyclic AMP produced by papaverine. For each experiment, $n = 5$ or more. Incubation of ganglia with 0.1 mM papaverine for 40 min increased cyclic AMP concentration from 17.83 ± 1.61 to 51.5 ± 6.51 ($P < 0.001$). However, when ganglia were incubated with 0.1 mM papaverine for 20 min followed by 0.1 mM papaverine plus 5 mM theophylline for 20 min, cyclic AMP increased to only 24.3 ± 1.9 ($P < 0.05$). *d*, Effect of theophylline on the % increase in cyclic GMP produced by papaverine. For each experiment, $n = 5$ or more. Incubation of ganglia with 0.1 mM papaverine increased cyclic GMP concentration from 0.98 ± 0.07 to 2.02 ± 0.17 ($P < 0.001$). However, when ganglia were incubated with 0.1 mM papaverine for 20 min followed by 0.1 mM papaverine plus 5 mM theophylline for 20 min, cyclic GMP increased to only 1.5 ± 0.19 ($P < 0.05$). Cyclic nucleotide content was determined as follows. After dissection, ganglia were equilibrated in oxygenated Ringer's solution for 1–2 h. After test procedures, ganglia were plunged into liquid nitrogen. Samples were fixed in 0.5 ml 0.1 M HCl in 90% methanol at -20°C for 2 h. Following addition of 1 ml water, samples were extracted at 80°C for 30 min and centrifuged. The supernatants were transferred, lyophilised, resuspended in water and assayed by radioimmunoassay using the method of Harper and Brooker¹⁵. Protein content was determined by the method of Lowry²⁴.

duration of the spike AH is thus important for determining the factors involved in regulating excitability and the frequency of spike firing. Further investigation of the potentiation of the spike AH by theophylline may lead to a greater understanding of the molecular mechanisms involved in the control of Ca^{2+} -sensitive K^+ conductances.

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Whitefly have the highest contraction frequencies yet recorded in non-fibrillar flight muscles

ASYNCHRONOUS (myogenic) insect flight muscles have evolved independently at least seven times¹. Although such muscles do not necessarily contract at particularly high frequencies, potentially they allow far greater wing stroke rates than synchronous muscle which, because of the delays involved in muscle activation, has been believed to be incapable of operating at frequencies above ~ 100 Hz (ref. 2), unless power output is small and much of the muscle volume occupied by sarcoplasmic reticulum³. Asynchronous muscle has so far always been shown to be of the 'fibrillar' histological type, and this correspondence has been assumed to be invariable¹. Predictably, very small insects, for which a high stroke frequency is believed to be essential², almost always have fibrillar flight muscle. However, a few groups, notably the hemipterous family Aleyrodidae (whitefly), have muscle of the 'close-packed' type, hitherto supposed to operate only synchronously¹. We have measured cinematographically the wing-beat frequencies of the whitefly *Trialeurodes vaporariorum* (Westwood), and report here that they are well above the usually assumed limit for synchronous functioning. Either this limit, or the assumption that all asynchronous muscle is of fibrillar type, must therefore be incorrect.

The studies of Greenwalt⁴ indicate that, albeit with many deviations, insect wing-beat frequencies vary approximately as the reciprocal of the wings' linear dimensions. Weis-Fogh has confirmed that within many families and superfamilies this is

almost exactly true, and has discussed the mechanical and aerodynamic reasons for it⁵.

The smallest flying insects which lack fibrillar muscle are probably some Neptulidae (Lepidoptera), with wing length 2 mm or less, and Aleyrodidae (Hemiptera), whose wings are often shorter than 1.5 mm, and of which *Trialeurodes vaporariorum* undoubtedly has close-packed flight muscle¹. Insects of this size might be expected to have wing-beat frequencies well above the assumed limit of 100 Hz for synchronous flight muscle. Various small Diptera with wings between 2 and 7 mm long show frequencies between 150 and 600 Hz (ref. 4), and psyllid, mirid and cimicid Hemiptera within the same size range have been timed at between 100 and 150 Hz (ref. 1). All these have fibrillar flight muscle. We have filmed *T. vaporariorum* in free flight, using a Hycam high-speed cine camera (Hadland), operating at approximately 3,000 frames per s. The exact framing rate was measured using a timing light generator operating a neon timing light within the camera which marked the film at precisely 1,000 Hz.

The flapping frequencies of nine individuals were measured during short flights. They varied between 143 and 181 Hz, that is between 40% and 80% higher than the previously supposed maximum contraction frequency for synchronous flight muscle.

The highest wing-beat frequencies previously recorded for insects using close-packed muscle are 90–95 Hz in some sphingid moths². *Trialeurodes*, by contrast, falls in the same frequency range as many medium-to-large Diptera and Hymenoptera⁴ and rather above those of most recorded Coleoptera⁴ and Heteroptera¹. Published figures for Aphididae, which like Aleyrodidae are Homoptera but which have fibrillar muscle, all fall below 100 Hz (ref. 1). Why asynchrony should have evolved in aphids but not in the usually smaller and more rapidly beating whitefly is unclear.

For insects of their size the wing-beat frequency of *Trialeurodes* is still strikingly low. Two factors may contribute to this. First, Pringle (ref. 2, text and subsequent discussion) has suggested that Aleyrodidae may be able to limit their frequency through their unusually low wing loading, which would reduce the minimum power necessary for flight. Second, Weis-Fogh⁵ explains the relatively low frequency of *Drosophila*—195–240 Hz in *Drosophila virilis*⁶—by their use of the 'clap and fling' mechanism⁷ for generating extra lift. *Trialeurodes* performs a dorsal clap and fling in each wing beat; also the wing loading, which we have calculated by dividing the mean weight of 800 individuals by the mean wing area of a sample of 15, is exceptionally low. Its value, 0.047 kg m⁻², may be compared with 0.35 kg m⁻² for the larger *D. virilis*⁶ and 0.086 kg m⁻² for the smaller *Encarsia formosa* (recalculated from Weis-Fogh⁷ using Ellington's figure for wing area⁸). Such a low wing loading will undoubtedly have contributed to a low power requirement, which may be a prerequisite for high-frequency synchronous muscle—if this is what *Trialeurodes* is using.

Contraction frequencies up to 180 Hz in close-packed flight muscle imply one of two possibilities. First, the previously assumed upper frequency limit for synchronous flight muscle contraction may be far too low. Alternatively, the asynchronous mechanism may not, after all, be restricted to fibrillar muscle. This certainly cannot be ruled out; many different kinds of muscle are now known to display the delayed stretch activation which is required for asynchronous operation. The crucial information now required is the stimulation frequency of the muscles. Without this it is impracticable to judge which explanation is correct.

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Antiserum specific for motor nerve terminals in skeletal muscle

OUR understanding of synaptic function and development has benefited from the availability of neurotoxins that bind specifically to acetylcholine receptors and acetylcholine esterase at the neuromuscular junction^{1–4}. It would be of additional value to have molecular probes for other components of this accessible and well-studied synapse, such as the nerve terminal. Antibodies to the synaptic vesicles that are concentrated in the motor nerve terminal would constitute one such marker; unfortunately, these vesicles are present in such small amounts that their purification has not yet been achieved. It is, however, possible to purify cholinergic synaptic vesicles from the electric organ of the elasmobranchs, *Narcine brasiliensis*⁵ and *Torpedo marmorata*⁶, and to obtain antisera to them (S.S.C. and R.B.K., in preparation). We report here that antibodies in anti-*Narcine* vesicle sera bind specifically to motor nerve terminals in skeletal muscle.

We have used the technique of indirect immunofluorescence to detect binding of antibodies to nerve terminals. Unfixed tissue (rat diaphragm except where noted) was frozen in liquid N₂ and sectioned in a cryostat. Sections were incubated with rabbit antiserum and then with a mixture of fluorescein-conjugated second antibody (goat anti-rabbit IgG) and rhodamine-conjugated α -bungarotoxin^{3,7}. Appropriate filter combinations enabled us to excite and view fluorescein or rhodamine selectively, and thus to compare the distribution of immunohistochemical stain with that of α -bungarotoxin, which binds tightly and specifically to the acetylcholine receptors that are clustered in the postsynaptic membrane of the neuromuscular junction^{1,2}.

Our main results are shown in Fig. 1. Unfractionated antisera to whole *Narcine* synaptic vesicles⁵ stained a variety of membranes in rat skeletal muscle, including those of blood vessels, nerves and muscle fibres (Fig. 1a). Most intensely stained, however, were small patches in the muscle fibres' surface (Fig. 1a), which could be identified as neuromuscular junctions because they also stained with rhodamine- α -bungarotoxin (Fig. 1b). Adsorption of antiserum with vesicle-free membranes from *Narcine* electric organ (S.S.C. and R.B.K., in preparation) removed antibodies that stained nonsynaptic membranes, but spared antibodies that stained the neuromuscular junction (Fig. 1c and d). This adsorbed antiserum also stained neuromuscular junctions in frog and chick skeletal muscle, and the innervated face of *Narcine* electropiaques. Adsorption of serum with sonicated *Narcine* vesicles reduced staining markedly. Preimmune serum did not stain neuromuscular junctions detectably. Thus, antibodies in anti-vesicle serum cross-react with components of mammalian, amphibian and avian neuromuscular junctions.

Two observations indicate that the neuromuscular components to which the antibodies bound were associated with nerve terminals. First, antibodies stained structures that resembled nerve terminals in size, shape and position^{8,9}: comparison of the distribution of fluorescein-antibody and rhodamine-toxin showed that antibodies stained 1–3 μ m wide structures (Fig. 1c, inset) that were tangential to toxin-stained gutters of post-synaptic membrane (Fig. 1d, inset). Second, antibodies did not

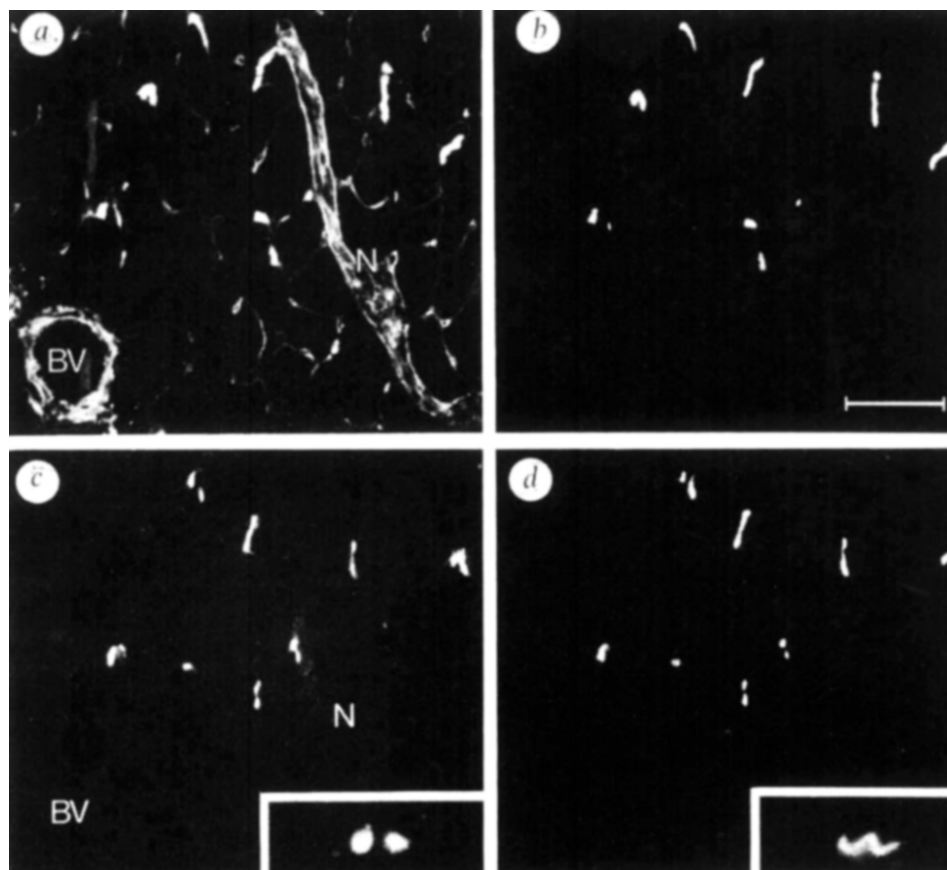


Fig. 1 *a, b*, Cross-section (4 μ m) of frozen, unfixed rat diaphragm were incubated for 30 min at room temperature with rabbit antiserum to *Narcine* synaptic vesicles (1:60 dilution in phosphate-buffered saline containing 1% BSA). They were then washed and stained for 30 min at room temperature with a mixture of fluorescein-'second antibody' (fluorescein-goat anti-rabbit IgG; Cappel Laboratories; 1:50 dilution) and rhodamine- α -bungarotoxin (10 nM; a gift of P. Ravdin and D. Berg, University of California, San Diego). The section was photographed with filters appropriate to show either fluorescein (*a*) or rhodamine (*b*). Immunohistochemical methods are detailed elsewhere¹¹. Fluorescein fluorescence reveals distribution of anti-vesicle antibodies and rhodamine the distribution of acetylcholine receptors^{3,7} and thus location of neuromuscular junctions. The coincidence of fluorescein and rhodamine shows that antibodies stain the neuromuscular junction; blood vessels (BV), nerves (N), and the muscle fibres' surface also stain. *c, d*, Section adjacent to that shown in *a* and *b*, incubated with anti-vesicle serum (1:60 dilution) that had been adsorbed with non-synaptic membrane from *Narcine* (5 μ g membrane protein/ μ l antiserum, 16 h, 4 °C), and then stained with fluorescein-second antibody and rhodamine- α -bungarotoxin: *c*, fluorescein; *d*, rhodamine. Neuromuscular junctions stain, but extrasynaptic portions of the muscle fibre surface do not. Blood vessel and nerve were stained too faintly to be visible in the micrograph, but their positions are marked. Insets to *c* and *d* show a single neuromuscular junction (from the section in *c* and *d*), photographed to show fluorescein (inset to *c*) or rhodamine (inset to *d*). Antibody stains nerve terminals that abut the toxin-stained postsynaptic membrane. The scale bar in *b* is 50 μ m and applies to *a-d*; 10 μ m for insets.

stain endplates in muscles that were denervated 1 week before they were frozen and sectioned. At this time, postsynaptic receptors and Schwann cells remain, but nerve terminals have disappeared¹⁰. There is, therefore, little doubt that antibodies bind to antigens in the motor nerve terminal or on its surface.

The antigens detected by the anti-vesicle serum are distributed non-uniformly in the motoneurone, as would be expected if they were associated with synaptic vesicles. Although nerve terminals stained intensely, axons in the nerve or in intramuscular nerve branches, cut either longitudinally or in cross-section, did not stain well (Fig. 1*c*), nor did the cytoplasm of motoneurone somata or proximal dendrites, seen in frozen sections of rat spinal cord. (The synapse-rich gray matter of the spinal cord did stain, however, raising the possibility that nerve terminals other than motor nerve terminals contain antigen; experiments to examine this possibility are in progress.)

In conclusion, antibodies directed against *Narcine* synaptic vesicles recognise motor nerve terminals in mammalian, amphibian and avian skeletal muscle, and distinguish terminal from preterminal portions of motor axons. These antibodies should facilitate studies of the function, development, and molecular composition of nerve terminals at the neuromuscular junction.

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T-cell-derived helper factor allows Lyt 123 thymocytes to differentiate into cytotoxic T lymphocytes

It is generally accepted that the diversity of T-cell responsiveness is generated in the thymus¹. It is also known that except for a few Lyt 1 cells all thymocytes express the Lyt 123 phenotype^{2,3}. Surprisingly, thymocytes are poorly responsive *in vitro*⁴, and only the medullary thymocytes, comprising 5–10% of the total thymic cell population, show an *in vitro* responsiveness comparable with that of peripheral T cells⁵. Cortical thymocytes, comprising 90–95% of all thymocytes, have previously been considered to be immature and immunologically incompetent⁴. The result reported here show that thymocytes are able to generate alloantigen-, virus- and hapten-specific cytotoxic T lymphocytes (CTL), provided a T-cell-derived helper factor is added to the cultures. Furthermore, through the use of cell separation techniques we conclude that in the presence of the helper factor the great majority of Lyt 123⁺ thymocytes have the capacity to differentiate into either alloreactive or H-2-restricted CTL.

The tissue culture system for the induction of alloreactive, trinitrophenyl (TNP)-specific and Sendai virus-specific CTL as well as the preparation of TNP-conjugated or Sendai virus-infected targets has been described elsewhere^{6–8}. The target cells used in the 3-h ⁵¹Cr-assay were *in vitro* propagated P815 (H-2^d) mastocytoma cells, LS cells (H-2^k) (Flow) and EL4 (H-2^b) tumour cells. Peanut agglutinin (PNA)-receptor-positive cells were detected using fluorescein isothiocyanate (FITC) labelled PNA (Industrie Biologique Française)^{9,10}. The preparation of semi-purified T-cell-derived helper factor has been described in detail elsewhere¹¹. Briefly, CBA mouse-derived splenic T cells were cultured in serum-free medium for 24 h in the presence of 1 µg ml⁻¹ concanavalin A (Pharmacia). The culture supernatant was concentrated 20-fold, eluted from a Sephadex G-100 column and the fractions with 'secondary CTL-inducing activity'¹¹ were pooled and concentrated 20-fold.

As can be seen in Table 1, CBA mouse (H-2^k)-derived splenic responder T cells are able to mount *in vitro* an efficient CTL response towards H-2^d alloantigens. Similarly efficient H-2-restricted CTL responses can be generated which are specific for TNP-conjugated or Sendai virus-coated syngeneic targets. In contrast, CBA mouse-derived thymocytes used as responder cells responded only poorly, if at all. However, the low responsiveness of thymocytes was converted to high responsiveness when an optimal concentration of T-cell-derived helper factor was added to the cultures (Table 1). The augmented CTL responsiveness of thymocytes was observed for the induction of both alloreactive CTL and H-2-restricted TNP- or Sendai virus-specific CTL.

These results support two broad conclusions. First, thymocytes are immunocompetent at the level of alloreactive and H-2-restricted CTL precursors. Second, a rate-limiting step during the *in vitro* induction of thymocyte-derived CTL can be bypassed by supplementing the cultures with T-cell-derived helper factor.

Thymocytes can be separated into two classes of cells. Medullary thymocytes, comprising 5–10% of thymocytes, are immunocompetent, have high H-2 and low Thy-1 density⁴ and are PNA-receptor negative¹⁰ and cortisone resistant⁵. In contrast, cortical thymocytes, which represent 90–95% of thymocytes, are PNA-receptor positive^{9,10}, have low H-2 and high Thy-1 density⁴ and are believed to be immunologically incompetent⁴. In view of the observed ability of thymocytes to generate CTL in the presence of T-cell-derived helper factor, we tested whether the use of cell separation techniques would allow us to define a particular subpopulation which responded in the culture conditions used.

CBA mouse-derived thymocytes were subject to 1g velocity

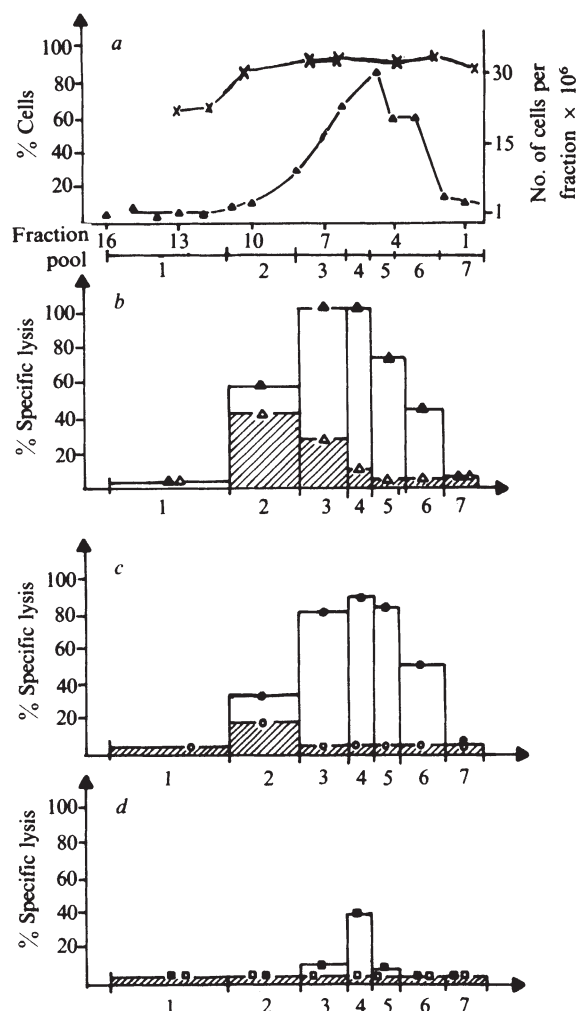


Fig. 1 CTL responsiveness pattern of thymocytes fractionated according to cell size. *a*, CBA mouse-derived thymocytes (2×10^8) were subjected to 1g velocity sedimentation using the Bont-De Koningh apparatus¹². One of the advantages of this apparatus is the short sedimentation time required to separate the cells to be fractionated. This is achieved by turning the sedimentation vessel from its vertical position into the horizontal position. The number of viable cells per individual fraction obtained is given. Note that fraction 1 contains the very small cells whereas fraction 15 contains the very large cells. $\times$ Indicates the % of cells binding FITC-conjugated PNA. *b*, Fractionated thymocytes (3×10^6) were cultured with 1×10^6 irradiated (1,000 rad) BALB/c stimulator cells in TC-24 Linbro culture plates in the absence (Δ) or presence ($\blacktriangle$) of 50 µl of semi-purified¹¹ T-cell-derived helper factor. After 5 d the lytic activity generated was tested in a 3-h ⁵¹Cr-assay against P815 target cells at a ratio of attacker to target cells of 20:1. Background lysis of P815 targets was 9%. *c*, Fractionated thymocytes (3×10^6) were cultured with TNP-conjugated CBA spleens in the absence ($\circ$) or presence ($\bullet$) of 50 µl semi-purified T-helper factor. After 5 d the lytic activity generated was assayed against TNP-conjugated LS cells at a ratio of CTL to target of 20:1. Background lysis was 12%. *d*, Fractionated CBA thymocytes (3×10^6) were cultured with 1×10^6 Sendai virus-coated syngeneic spleen cells in the absence ($\square$) or presence ($\blacksquare$) of 50 µl T-cell-derived helper factor. After 5 d the lytic activity generated was tested against Sendai virus-coated LS target cells at a ratio of CTL to target of 20:1. Background lysis was 17%. A similar responsiveness pattern was noted with fractionated BALB/c (H-2^d) and C57 BL/6 (H-2^b) thymocytes, the only variable being that in the absence of T-helper factor no response was obtained.

sedimentation using the Bont-De Koningh separation apparatus¹². Viable cells of the individual fractions obtained were tested for PNA-positive cells and for their CTL responsiveness to H-2^d stimulator cells, TNP-conjugated syngeneic stimulator cells and Sendai virus-coated syngeneic cells.

Figure 1 shows the results of a representative experiment. As can be seen from the cell distribution profile, thymocytes could be separated on the basis of cell size. PNA-negative cells were found only in the medium-to-large sized cell fractions. Cells collected in fractions 1–8 were PNA positive. With regard to

Table 1 Effect of T-cell-derived helper factor on the capacity of CBA mouse thymocytes to mount alloreactive and H-2-restricted CTL responses

Responder cells (H-2 ^a)	X-ray irradiated splenic stimulator cells	Semi-purified helper factor added (μ l per culture)	⁵¹ Cr-labelled target cells	% Specific lysis (ratio of CTL to target cells)		
				10:1	2:1	0.5:1
CBA thymocytes	BALB/c (H-2 ^d)	—	P815	7	3	0
	BALB/c	75	P815	90	77	44
	BALB/c	25	P815	61	49	17
CBA thymocytes	CBA-TNP	—	LS-TNP	2	1	0
	CBA-TNP	75	LS-TNP	67	45	9
	CBA-TNP	25	LS-TNP	26	9	2
CBA thymocytes	CBA-Sendai virus	—	LS-Sendai virus	0	0	0
	CBA-Sendai virus	75	LS-Sendai virus	32	12	4
	CBA-Sendai virus	25	LS-Sendai virus	17	9	2
CBA spleen	BALB/c spleen	—	P815	98	57	32
	CBA-TNP	—	LS-TNP	63	27	9
	CBA-Sendai virus	—	LS-Sendai virus	44	18	6

CBA mouse-derived responder cells (4×10^6) were cultured in the presence of the respective X-irradiated (1,000 rad) splenic stimulator cells (1×10^6) in 2 ml of medium in Linbro FB-24 Tc multidish culture trays (Linbro). Cultures contained 5% fetal calf serum, 10 mM HEPES, 2×10^5 M 2-mercaptoethanol with or without T-cell-derived helper factor¹¹. After 5 d incubation at 37 °C, the cells were collected and set up in a standard 3-h ⁵¹Cr-release assay against the target cells listed. The preparation of target cells using 10 mM 2,4,6-trinitrobenzene sulphonic acid (Sigma) of β -propiolactone-inactivated Sendai virus suspension (Lot no. 138-1, Connaught) has been described elsewhere⁸. Background lysis of the target cells did not exceed 17%; % specific lysis represents the mean of triplicate determinations. In no case did the standard deviation exceed 5%.

cytolytic activity, medium-to-large sized thymocytes were able to mount CTL responses when stimulated *in vitro* with allogeneic stimulator cells, but were less responsive when stimulated with TNP-conjugated cells, and were unresponsive to stimulation with Sendai virus-coated syngeneic cells (Fig. 1). The unexpected finding, however, was that the apparent immunoincompetence of the large cell pool of medium-to-small sized thymocytes was converted to a state of high responsiveness when the T-cell-derived helper factor was added to the cultures. Interestingly, in the presence of this factor medium-to-small sized thymocytes not only mount efficient CTL responses towards alloantigens, but are also able to generate H-2-restricted CTL responses for the hapten TNP. Even Sendai virus-specific CTL could be induced from thymocytes of pool 4. The data in Fig. 1 also show that the very large and very small thymocytes were unable to generate CTL. In this context it is noteworthy that medium-to-small thymocytes were all PNA positive. This marker has recently been attributed to cortical thymocytes¹⁰, known to express the Lyt 123 phenotype³. We therefore conclude that in the conditions described cortical thymocytes can differentiate into alloreactive as well as H-2-restricted CTL.

The experiments described formally demonstrate that depending on the 'antigen' used, thymic Lyt 123 T cells have the potential to give rise to H-2-restricted TNP or virus-specific CTL, or to non-restricted alloreactive CTL. The results also suggest that within cortical thymocytes both alloreactive and H-2-restricted CTL precursors express the Lyt 123 phenotype, and that at least at the level of CTL precursor cells, cortical thymocytes already express an antigen receptor diversity comparable with that of peripheral T cells. Obviously, at the level of CTL precursors cortical thymocytes are immunocompetent, and have the potential to respond efficiently to either alloantigens or foreign antigens. Thus, if the cortex of the thymus represents the site for the generation of the T-cell repertoire, immunocompetent cortical thymocytes are the result of these events. It should be stressed that *in vitro* the expression

of the immunocompetence of cortical thymocytes occurs only in the presence of a helper factor previously shown to be derived from activated Lyt 1 T cells¹¹. In fact, *in vitro* the rate-limiting step for the differentiation of Lyt 123 thymocytes into CTL represents the presence of the T-cell derived helper factor (Fig. 1).

Although the mechanism of this T-T-cell interaction is unknown, our experiments raise the question as to why within peripheral T cells of adult mice alloreactive T cells develop from the Lyt 23 T-cell subset². Because, within the thymus, Lyt 123 T cells apparently represent the precursor pool of alloreactive CTL, a shift from Lyt 123 to the Lyt 23 T cell must take place. Recently, Burakoff *et al.*¹³ provided convincing evidence that in mice within 3 weeks of birth the phenotype of peripheral alloreactive CTL precursors changes from Lyt 123 to Lyt 23. As the Lyt 23 phenotype is characteristic of H-2-restricted CTL, alloreactive CTL and memory T cells (refs 14–16 and unpublished results), and as H-2-restricted CTL specific for 'foreign antigens' to cross-react with alloantigen^{17–19}, we postulate that alloreactive peripheral Lyt 23 CTL precursors represent memory cells of virus-specific H-2-restricted CTL which by chance cross-react with alloantigen. A prediction of this view is that H-2-restricted versus non-restricted cytotoxicity is not a function of CTL, but a result of the respective target antigen recognised. In accordance with this we recently defined an *in vitro* propagated monoclonal CTL line (H-2^a) specific for allogeneic K^d targets. These monoclonal CTL also lyse (unpublished results) Sendai virus-infected syngeneic cells in an H-2-restricted manner.

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Lymphomas with cytotoxic activity

TUMOURS of antibody-secreting B lymphocytes (myeloma tumours) have contributed greatly to our understanding of antibody structure and function. T-lymphocyte tumours that retain effector activities might, if they exist, prove to be similarly useful for analysing T cells and their key components. The recent finding that a T lymphoma is capable of crawling on other cells, like antigen-activated normal T lymphocytes¹, prompted

Table 1 Con A-dependent cytotoxicity of some mouse lymphomas

	Origin				
Lymphoma	Strain (H-2 haplotype)	Tissue*	Induction†	Ref.	Cytotoxicity (% specific lysis)
	Positive control: BALB/c anti-EL-4				27.3 ± 0.1
TB-1	AKR (k)	LN	Spont.	3	14.4 ± 1.2
TB-2	AKR (k)	Spl	Spont.	3	17.8 ± 1.5
TB-3	AKR (k)	LN	Spont.	3	20.7 ± 0.7
Ib	C58 (k)	Spl	Spont.	14	0.1 ± 1.2
UM-1	C58 (k)	Spl	Spont.	‡	0.4 ± 0.1
UM-2	C58 (k)	Spl	Spont.	‡	1.0 ± 0.2
CAM-1	BALB/c (d)	Spl	Spont.	‡	0.2 ± 1.3
A13	BALB.B (b)	LN, Spl	MuLV	15	-0.1 ± 0.8
10B89	B10 × BALB.B (b)	LN, Spl	MuLV	15	1.5 ± 1.6
DC75	B10.D2 × BALB/c (d)	LN, Spl	MuLV	15	0.3 ± 0.9
F39	SWR/J (s)	LN, Spl	MuLV	‡	0.3 ± 0.3
PF6-MELN	SWR/J (s)	LN	MuLV	‡	0.2 ± 0.6
L1210	DBA/2 (d)	LN, Spl	MCA	16	0.2 ± 1.0
P288	DBA/2 (d)	LN	MCA	17	0.2 ± 1.2
P388	DBA/2 (d)	LN	MCA	18	1.0 ± 0.8
L5178Y	DBA/2 (d)	Spl	MCA	19	0.2 ± 1.3
Lymphoma 4	DBA/2 (d)			20	-0.5 ± 0.8
SL-2	DBA/2 (d)		Spont.	21	0.8 ± 0.1
P1534	DBA/2 (d)	Thymus	Spont.	22	0.9 ± 0.4
R1	C58 (k)	Thymus	Spont.	23	-0.4 ± 1.2
EL-4	C57Bl/6 (b)	Thymus	DMBA	24	-0.7 ± 1.6
AKR-3T	AKR (k)	Thymus	Spont.	‡	-1.0 ± 0.3
RLδ1-11	BALB/c (d)	Thymus	X-ray	25	1.0 ± 0.4
L691-6	C57L (b)	Thymus	X-ray	26	0.3 ± 1.4

Per cent specific target cell lysis is defined as $100(ER - SR)/(100 - SR)$, where ER (experimental release) and SR (spontaneous release) are percentages of ^{51}Cr release from target cells in, respectively, the presence and absence of Con A. Values are mean ± s.d. of determinations at lymphoma to target cell ratios of 13 and 25. The presence of lymphoma cells slightly (1 or 2%) decreased the spontaneous release of ^{51}Cr from target cells (in the absence of Con A). The target cells, MOPC-167 cells (H-2^d), were labelled by incubation in medium containing $\text{Na}_2^{51}\text{CrO}_4$, and cytotoxicity was assayed as described¹³. Cytotoxic cells used as positive controls for the Con A-dependent cytotoxicity were peritoneal exudate cells taken from BALB/c (H-2^d) mice that had two intraperitoneal injections of EL-4 (H-2^b) cells¹, that is, H-2^d anti-H-2^b. TB-1 and TB-3 arose as spontaneous lymph node lymphomas in old (>14 months) thymectomised AKR mice; TB-2 arose as a spontaneous spleen lymphoma in a 14–16-month-old AKR mouse with an atrophic thymus³. These three lymphomas were adapted to grow in culture by Durda, Boos and Gottlieb²⁷. Ib and UM-2, and CAM-1 are maintained in C58 and BALB/c mice, respectively, by intraperitoneal passage of cells from enlarged spleens. The seven DBA/2 tumours are maintained in ascites tumours in DBA/2 or CD2 F₁ mice. All other lymphomas are maintained in culture.

* Source from which the lymphoma was derived, not necessarily the tissue in which neoplastic transformation took place. LN = lymph node; Spl = spleen.

† Spont. = spontaneous; MuLV = murine leukaemia virus; DMBA = dimethyl benzanthracene; MCA = methylcholanthrene.

‡ UM-1 and UM-2 were isolated by L. Pease and W. H. Murphy, CAM-1 by T. W. Chang, E. Celis and H. N. Eisen, F39 and PF6-MELN by P. Fahnestock, N. H. Hopkins and P. D. Gottlieb, and AKR-3T by N. Rosenberg.

us to investigate whether some lymphocytic tumour cells can, like normal cytotoxic T lymphocytes, lyse target cells. We report here that they can.

Because it was unlikely that any particular cytotoxic T lymphoma would be specific for any of the few target cells available to us, we included concanavalin A (Con A) in the assay mixture of lymphoma cells and target cells. In the presence of Con A cytotoxic T cells lyse target cells nonspecifically, evidently because Con A links killer and target cells and activates the killer cell's lethal mechanism². Initially we used cells of a myeloma (MOPC-167) of BALB/c mice as targets.

In a typical assay, 1×10^4 ^{51}Cr -labelled MOPC-167 cells and 0 to 50×10^4 lymphoma cells were mixed in a total volume of 200 μl of a standard cell culture medium (RPMI-1640 with 10% fetal calf serum), with or without Con A ($5 \mu\text{g ml}^{-1}$). The cells were incubated at 37 °C for 4 h, and the ^{51}Cr released into the medium by the target cells was measured. A positive control was included in each assay: either peritoneal exudate lymphocytes from BALB/c mice that had been immunised against allogeneic EL-4 lymphoma cells of C57Bl/6 mice¹, or one of the lymphomas with cytotoxic ('killer cell') activity (see below).

None of the 24 lymphomas tested was cytotoxic in the absence of Con A, but three (TB-1, TB-2 and TB-3) had cytotoxic activity in the presence of Con A (Table 1). Representative titrations are shown in Fig. 1. The three active lymphomas also caused Con A-dependent lysis of other target cells (P815, a mastocytoma of the DBA/2 strain, and EL-4), and their cytotoxic activity was facilitated just as well by phytohaemagglutinin as by Con A (data not shown).

The surface antigens of TB-1, TB-2 and TB-3 were previously studied with *in vivo*-passaged tumours and with TB-1 as cultured cells³. In addition to Thy-1 and Lyt, characteristic T-cell markers⁴, these tumours were observed, through the use of immunofluorescence, intermittently to have surface (but not intracellular) immunoglobulins, Ia, and Fc receptor, considered characteristics of B cells⁵. We verified that virtually all cells of the cytotoxic cultured lines used in the present study could be lysed specifically by anti-Thy-1.1 antibody⁶ (plus complement); we are re-investigating whether they synthesise immunoglobulin. The Lyt antigens and the crawling activity of all the tumours listed in Table 1 will be reported elsewhere (T.W.C., E. Celis, P. D. Gottlieb, F. Solomon and H.N.E., in preparation). Tentatively, it seems that all the killer cells have crawling activity, but that some crawlers are not cytotoxic¹.

Previous studies indicate that lysis of target cells in the presence of Con A or phytohaemagglutinin is due to cytotoxic T cells, not to other activated T cells⁷, and that the same lytic mechanisms are involved in Con A-dependent and antigen-dependent target cell lysis⁸. Other evidence, however, indicates that the T cells engaged in Con A-dependent killing may lack specific receptors for antigen on target cells, while retaining the killing apparatus⁹. If specific receptors are absent, it may be possible to introduce them by fusing the immortal killer lymphomas with normal cytotoxic T lymphocytes of known specificity (or their precursors), just as myeloma cells have been fused with normal B lymphocytes to yield hybridomas that produce specific antibodies¹⁰. Cytotoxic T lymphomas are potentially useful for studying the cytolytic mechanism of killer lymphocytes and the properties of their antigen-recognition molecules.

Most mouse T lymphomas arise in the thymus, but the three cytotoxic lymphomas of Table 1 originated in the spleen or lymph nodes³. It may be more useful to look for additional killer T lymphomas among the rare lymphomas that seem to arise in relatively mature T-cell populations of spleen and lymph nodes than among the more common lymphomas that arise in the thymus. Lymphomas seem to appear with relatively high frequency in spleen and lymph nodes in old (>12 months) mice of the C58, B10.Y and HRS/J strains (H. Meier and W. B. Murphy, personal communications). Normal cytotoxic T cells, maintained in culture for long periods, are being evaluated as alternative sources of homogeneous killer cells. However, these normal cells require restimulation of T-cell factors for growth and for generation of cytolytic activity^{11,12}, and the quantity of cells that can be obtained is limited.

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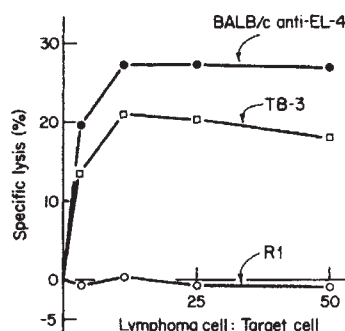


Fig. 1 Con A-dependent lysis of target cells by normal cytotoxic T lymphocytes and two lymphomas. The targets, immune normal lymphocytes, and lymphomas are described in Table 1.

lines: P. Gottlieb (TB-1, TB-2, TB-3, F87N, PF6-MELN, A13, 10B89, DC75), W. Murphy (Ib, UM-1, UM-2), M. Bevan (R1), H. Winn (EL-4), N. Rosenberg (AKR-3T, L691-6), E. Siden (RL3-11), and B. Benacerraf (SL-2); Arthur D. Little Co. provided P288, P388, L1210, P1534, L5178Y and Lymphoma 4. This work was supported by grants CA-15472 and CA-14051 from the NCI, DHEW, and by grant IM-161 from the American Cancer Society.

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The role of glucocorticoid receptor and gene expression in the anti-inflammatory action of dexamethasone

GLUCOCORTICOIDS are potent anti-inflammatory agents, but the molecular mechanisms involved in this activity are not yet understood. The biological actions of various steroid hormones have been shown to be mediated by functional proteins synthesised following translocation of a hormone-cytoplasmic receptor complex into the nucleus^{1,2}. It is still not clear, however, whether the anti-inflammatory effects of glucocorticoids are mediated via binding to a cytoplasmic receptor in target cells and subsequent activation of gene expression³, although glucocorticoid receptors have been demonstrated in HTC cells, liver⁵, thymocytes⁶ and lymphocytes⁷. The results reported here suggest that a glucocorticoid receptor and the induction of gene expression are involved in the anti-inflammatory action of dexamethasone.

Experiments were undertaken to determine (1) whether progesterone, fluoxymesterone and 17 α -methyltestosterone (designated by Samuels and Tomkins⁸ as 'anti-inducers' on the basis of their antagonism of corticoid induction of tyrosine aminotransferase in hepatoma tissue culture cells) interfere with anti-inflammatory action of dexamethasone and (2) whether RNA and protein synthesis is essential for the manifestation of the anti-inflammatory action of dexamethasone. Serotonin-induced footpad oedema in mice was used as a model of inflammation, since the mechanism of the vascular permeability

increase in serotonin-induced inflammation has been well characterised⁹.

An inflammatory response was induced by injecting serotonin (0.3 μ g in 5 μ l of pH 7.4 Tyrode's solution) in the subplantar region of the right foot of 6-week-old male ddY mice (25-30 g). The left foot was injected with Tyrode's solution only. Inflammatory response (oedema) was evaluated by measuring the difference in footpad thickness between right and left feet with a dial guage calliper 12 min after serotonin injection. The serotonin dose used was chosen on the basis of a dose-response curve obtained in preliminary experiments. Dexamethasone dissolved in ethanol/0.9% NaCl (1:39, v/v) was injected subcutaneously (s.c.) in the abdomen. Anti-inducer steroids were injected s.c. at a separate site on the abdomen.

Preliminary experiments suggested the existence of a time lag for manifestation of the anti-inflammatory effect of dexamethasone. To determine the appropriate dose for dexamethasone as inhibitor of serotonin-induced inflammation, dexamethasone was given at 0.15, 0.5 and 1.5 mg per kg body weight. There was significant inhibition of inflammation at 0.5 and 1.5 mg per kg (65 and 78% inhibition respectively). Using a dexamethasone dose of 0.5 mg per kg the inflammatory lesion was induced at various times after dexamethasone treatment. Dexamethasone did not inhibit serotonin-induced inflammation within 1 h after administration, but the anti-inflammatory effect was apparent at 2 h, reached a maximum at 3 h and persisted for 6 h or longer. Based on these results, the inflammatory lesion was provoked in all of the following experiments 2.5-6 h after injection of dexamethasone.

In the first series of experiments for anti-inducer compounds (Table 1), the test compounds were given 1 h before dexamethasone and footpad inflammation was provoked 3 h after dexamethasone injection. A single treatment with dexamethasone caused a marked reduction of the inflammatory response. Progesterone, fluoxymesterone and 17 α -methyltestosterone, when applied before dexamethasone treatment, brought about a marked inhibition of the anti-inflammatory effect of dexamethasone. The degree of the inhibition was dose-dependent, and complete blockade of the anti-inflammatory action was observed with all three anti-inducer steroids at doses of 200 mg per kg. Treatment with these non-corticoid anti-inducer steroids alone exerted no appreciable influence on the serotonin-induced inflammatory response. On the other hand, androstenedione, was classified as an inactive compound by Tomkins *et al.*⁸ exerted no detectable influence on either the inflammatory response to serotonin or the anti-inflammatory action of dexamethasone (Table 1, series 1).

Similarity between the ability of the anti-inducer steroids to block anti-inflammatory action of dexamethasone and their ability to inhibit both glucocorticoid induction of tyrosine aminotransferase and glucocorticoid binding with the specific receptor in HTC cells suggests that similar mechanisms may be involved in these processes. If binding of glucocorticoid to specific receptor, and subsequent transfer of the corticoid-receptor complex into the nucleus are essential components of anti-inflammatory corticoid effects, the application of an inhibitor of corticoid-receptor binding after the corticoid-receptor complex has been transferred into nucleus would not block the final effects of the corticoid. To examine such possibilities, the second series of experiments was performed. An anti-inducer steroid 17 α -methyltestosterone was given 1 h after dexamethasone treatment and serotonin was injected 3 h after dexamethasone to induce inflammation. The results are presented in Table 1 (series 2). Administration of the anti-inducer steroid at this late stage exerted no detectable influence on the anti-inflammatory action of dexamethasone. Thus 17 α -methyltestosterone inhibits glucocorticoid action by blocking exclusively an early step in the series of reactions which leads to the anti-inflammatory effect. Antagonism of some steroidal compounds to various biological effects of the corticoids has been described in HTC cells^{4,8}, skeletal muscle¹⁰ and thymocytes⁶, and discussed in relation to their ability to

Table 1 Effect of progesterone, fluoxymesterone, 17 α -methyltestosterone and androstenedione on anti-inflammatory action of dexamethasone in mice

										Time of treatment or measurement (h)
Series 1										
Progesterone (mg per kg)	0	0			100	100	200	200	-1	
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0	
Oedema (mm)	0.38±0.034	0.13±0.009*			0.25±0.013*†	0.36±0.032†	0.32±0.021†	0.32±0.034†	3	
Fluoxymesterone (mg per kg)	0	0			100	100	200	200	-1	
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0	
Oedema (mm)	0.35±0.024	0.10±0.006*			0.25±0.011†	0.31±0.033†	0.34±0.029†	0.37±0.031†	3	
17α-methyltestosterone (mg per kg)	0	0	50	50	100	100	200	200	-1	
Dexamethasone (mg per kg)	0	0.5	0.5	0	0.5	0	0.5	0	0	
Oedema (mm)	0.39±0.037	0.15±0.019*	0.19±0.035*	0.39±0.022†	0.29±0.041†	0.33±0.031†	0.33±0.049†	0.31±0.036†	3	
Androstenedione (mg per kg)	0	0			100	100	200	200	-1	
Dexamethasone (mg per kg)	0	0.5			0.5	0	0.5	0	0	
Oedema (mm)	0.41±0.028	0.15±0.013*			0.16±0.020*	0.36±0.024†	0.15±0.019*	0.34±0.015†	3	
Series 2										
17α-methyltestosterone (mg per kg)	0	0			100	100			1	
Dexamethasone (mg per kg)	0	0.5			0.5	0			0	
Oedema (mm)	0.41±0.041	0.09±0.007*			0.10±0.011*	0.36±0.031†			3	

In series 1 experiments, non-corticoid steroids were given s.c. 1 h before s.c. injection of dexamethasone. Serotonin-footpad-oedema was provoked 3 h after the dexamethasone treatment. In series 2, 17 α -methyltestosterone was injected s.c. 1 h after application of dexamethasone. Serotonin-footpad oedema was induced 3 h after the dexamethasone treatment. The oedema was expressed in terms of the increase in the footpad thickness and the values represent means of seven or eight mice $\pm$ s.e.m.

*Statistically significant differences with respect to control group, $P < 0.01$.

†Significance differences with respect to dexamethasone group, $P < 0.01$.

Table 2 Effect of cycloheximide and actinomycin D on anti-inflammatory action of dexamethasone in mice

									Time of treatment or measurement (h)
Series 1									
Dexamethasone (mg per kg)	0	0.5	0.5	0					0
Cycloheximide (mg per kg)	0+0	0+0	3+3	3+3					0 and 1.5
Oedema (mm)	0.47±0.026	0.17±0.016*	0.40±0.036†	0.49±0.042†					3
Dexamethasone (mg per kg)	0	0.5	0.5	0					0
Cycloheximide (mg per kg)	0+0	0+0	6+6	6+6					0 and 1.5
Oedema (mm)	0.45±0.058	0.16±0.025*	0.43±0.038†	0.38±0.041†					3
Dexamethasone (mg per kg)	0	0.5	0.5	0	0.5	0			0
Actinomycin D (mg per kg)	0+0	0+0	1+1	1+1	2+2	2+2			0 and 1.5
Oedema (mm)	0.38±0.033	0.14±0.024*	0.38±0.034†	0.39±0.072†	0.38±0.012†	0.45±0.029†			3
Series 2									
Dexamethasone (mg per kg)	0	0.5	0.5	0					0
Cycloheximide (mg per kg)	0+0	0+0	3+3	3+3					3 and 4.5
Oedema (mm)	0.36±0.043	0.06±0.012*	0.13±0.021*	0.39±0.055†					6
Series 3									
Dexamethasone (mg per kg)	0	0.5	0.5	0					0
Cycloheximide (mg per kg)	0	0	3	3					1
Oedema (mm)	0.47±0.058	0.15±0.015*	0.44±0.036†	0.41±0.036†					2.5

In series 1, cycloheximide and actinomycin D were given s.c., twice, simultaneously and 1.5 h after s.c. injection of dexamethasone. Serotonin-footpad oedema was provoked 3 h after the injection of dexamethasone. In series 2, cycloheximide was applied, twice, at 3 and 4.5 h after the application of dexamethasone and the serotonin oedema was induced 6 h after the dexamethasone injection. In series 3, cycloheximide was given 1 h after the dexamethasone treatment and the serotonin oedema was induced 2.5 h after the dexamethasone injection. Amounts of cycloheximide and actinomycin D used were sufficient to inhibit RNA and protein synthesis¹¹⁻¹³.

*Statistically significant differences with respect to control group, $P < 0.01$.

†Statistically significant differences with respect to dexamethasone group, $P < 0.01$.

compete with glucocorticoids in binding with the specific receptor in target cells. Our demonstration of antagonism by 'anti-inducer steroids' of the anti-inflammatory action of the corticoid suggests a role for corticoid receptor in the anti-inflammatory action of the corticoid. It will be difficult, however, to directly demonstrate antagonism at the receptor site because it is not clear which are the primary target cells in the anti-inflammatory action of the corticoid.

To test for the requirement for RNA and protein synthesis, actinomycin D or cycloheximide¹¹⁻¹³ was given twice, simultaneously with dexamethasone and 1.5 h later, in an attempt to inhibit RNA or protein synthesis for 3 h consecutively after the application of dexamethasone. The results summarised in Table 2 (series 1) clearly indicate that the anti-inflammatory effect of dexamethasone is completely blocked by actinomycin D or cycloheximide, suggesting a requirement for synthesis of new

RNA and protein. To establish whether uninterrupted protein synthesis throughout experimental periods is essential, mice were injected with cycloheximide 3 and 4.5 h after application of dexamethasone in an attempt to block protein synthesis for the period from 3 to 6 h after dexamethasone treatment. The inflammatory lesion was provoked at 6 h after the dexamethasone treatment. Cycloheximide given at this stage did not block the anti-inflammatory action of the steroid (Table 2, series 2). Further experiments were performed to characterise the mechanism of the effect of cycloheximide injected 1 h after application of dexamethasone: When serotonin footpad oedema was induced 1.5 h after application of cycloheximide there was complete blockade of the anti-exudative effect of dexamethasone (Table 2, series 3).

For several of the metabolic effects of glucocorticoids a latency period of 0.5–2 h has been reported¹⁴. These time lags probably represent time required for events subsequent to entering of the corticoid into target cells to bring about production of specific protein through gene expression. A similar time lag has been reported¹⁵ for the inhibitory effect of dexamethasone against experimental anaphylaxis and histamine-induced inflammatory reactions in rat and mice. In agreement with these observations, a latency period of 1–2 h was observed in the present experiments with respect to manifestation of the anti-inflammatory action of dexamethasone. When actinomycin D or cycloheximide was applied simultaneously (Table 2, series 1) with dexamethasone or during the latency period (Table 2, series 3), the anti-inflammatory effect of the steroid was abolished. Therefore, both RNA and protein synthesis appear to be essential for manifestation of the anti-inflammatory activity of dexamethasone. However, as the anti-inflammatory effect of the steroid was not affected by cycloheximide applied 3 h after administration of the steroid (Table 2, series 2), it is not essential that protein synthesis proceeds without interruption over the entire period. It is likely, therefore, that new RNA and protein synthesised under the influence of dexamethasone play an essential role in the manifestation of the anti-inflammatory activity.

During preparation of this paper Flower and Blackwell reported evidence of induction in the guinea pig lung by glucocorticoids of biosynthesis of a protein (or peptide) inhibitor which prevents prostaglandin generation¹⁶. It is possible that similar mechanisms might underlie the inhibitory action of dexamethasone against serotonin-induced inflammatory response, since serotonin stimulates some tissues to generation of rabbit aorta contracting substances (a mixture of prostaglandin endoperoxides and thromboxane A₂)¹⁷. However, the finding that indomethacin does not affect serotonin-induced paw oedema in rats¹⁸ suggests that serotonin provokes an inflammatory response independently of the prostaglandin and thromboxane generation system. We also confirmed that indomethacin was inert in inhibiting mice footpad oedemata induced by various phlogistic agents such as serotonin, bradykinin and PGE₁, whereas dexamethasone suppressed all of them. It is therefore likely that dexamethasone displays anti-inflammatory action, apart from phospholipase inhibition¹⁶, through direct suppression of endothelial cell contraction induced by chemical mediators such as serotonin⁹.

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A new proposal regarding the subunit composition of (Na⁺ + K⁺)ATPase

THREE types of high affinity ligands are available for studies with (Na⁺ + K⁺)ATPase: nucleotide substrates, vanadate and ouabain. Of these, the last is unique in apparently being absolutely specific for (Na⁺ + K⁺)ATPase. One of the generally accepted facts regarding (Na⁺ + K⁺)ATPase is that there are equal numbers of measurable (high affinity) binding sites for ATP and ouabain per molecule of enzyme^{1–5}. There is a majority opinion that direct binding experiments yield binding capacities for these ligands of 1 mol site per 250–300 kg protein in the purest preparations available^{3,6–8}. From these data, from determinations of the peptide composition and from estimates of the apparent molecular weight of solubilised enzyme the idea of (Na⁺ + K⁺)ATPase as a dimer (with respect to the large, so-called catalytic, peptide) has emerged^{9–14}. Such a dimer would have one high affinity binding site for nucleotide and one for ouabain. The present report on binding site determinations on mammalian kidney microsomes before and after detergent treatment casts serious doubts on the general validity of the above simple relationship between sites and peptide composition.

The essential points of this report are represented in Figs 1 and 2. Figures 1 and 2b show that, in agreement with what has been outlined above, detergent-treated kidney microsomes have an equal number of high affinity nucleotide-binding sites and of ATP-supported ouabain-binding sites and P_i-supported ouabain-binding sites. The number of vanadate-binding sites happens also to be the same, contrary to the findings of Cantley *et al.*⁵.

If one turns to the source of these enzyme preparations, that is, crude microsomal fraction from pig kidney outer medulla, it can be seen that this simple stoichiometry no longer holds (Table 1 and Fig. 2a). The microsomes have equal capacities for binding nucleotide and vanadate and for ATP-supported ouabain binding, but capacities exactly twice as large for both P_i-supported ouabain binding and for vanadate-supported ouabain binding.

The complexity of the situation is further stressed by the curvature of the isotherms for nucleotide binding, vanadate binding, and ATP-supported ouabain binding (Fig. 2a). Such curvature is diagnostic of an inhomogeneity in the site populations^{21,22}. On the assumption that the observed inhomogeneity is due to a mixture of only two populations of sites, non-linear regression analysis of the data²³ strongly suggests that for all three ligands the two populations are of identical size (Table 1). This result is also mathematically compatible with a system of two inherently identical, negatively cooperating sites. The excellent fit of the calculated isotherms to the experimental data, as seen in Fig. 2a, makes it unnecessary to consider more complicated models at this point.

Obviously, some changes have occurred in the microsomal enzyme during or subsequent to detergent treatment. Figure 2b shows the results of binding studies made in the presence of those amounts of SDS added to the same microsomal

Table 1 Binding capacities for various ligands and K^+ -dependent *p*-nitrophenyl phosphatase activities of microsomal ($Na^+ + K^+$)ATPase from pig kidney outer medulla before and after SDS treatment

Enzyme preparation	P _i -supported ouabain-binding capacity	Relative ligand binding capacities*			Relative <i>p</i> -nitrophenyl phosphatase activity [†]	Mol <i>p</i> -nitrophenyl phosphate split per min per mol P _i -supported ouabain-binding site
		Vanadate-supported ouabain-binding capacity	Nucleotide-binding capacity, vanadate-binding capacity, and ouabain-binding capacity (ATP-supported)			
			Total	Subpopulations		
Microsomes	4	4.0	2.0	1.07 & 0.93	1	0.39 × 10 ³
		4.0	2.0	0.96 & 1.04		0.41 × 10 ³
Microsomes + SDS	4.1	—	4.0	None apparent	3.9	1.53 × 10 ³
	3.9		4.0			1.59 × 10 ³
Purified enzyme preparations						1.49 × 10 ³
						1.52 × 10 ³

The table shows values obtained with two separate microsome preparations before and after addition of SDS.

* Values are expressed relative to P_i -supported ouabain-binding capacity of untreated microsomes. This we define as being equal to 4.

† Relative to that of untreated microsomes.

preparation of Fig. 2a so as to yield maximal *p*-nitrophenyl phosphatase activity. It can be seen (see also Table 1) that the SDS-treated microsomal enzyme has equal binding capacities for each of the ligands tested; that the P_i -supported ouabain-binding capacity has not changed; that *p*-nitrophenyl phosphatase activity has quadrupled; and that the molar activity of the phosphatase is identical to that of enzyme purified through SDS treatment. In other words, the enzyme has now been converted to the same state as found in purified (and pure) preparations. And pure preparations of ($Na^+ + K^+$)ATPase contain one ouabain-binding site and one nucleotide-binding site per two large, catalytic peptides. As the P_i -supported ouabain-binding capacity of the enzyme remains unchanged with SDS treatment, it must be concluded that untreated microsomes have one P_i -supported ouabain-binding site per two large peptides.

As microsomes have one nucleotide-binding site and one vanadate-binding site per two P_i -supported ouabain-binding sites, two conclusions are possible at this stage. (1) In microsomes there exists a mixture of exactly equal amounts of two types of enzyme, each containing two large peptides and one P_i -supported ouabain-binding site. One nucleotide-binding site and one vanadate-binding site must then be distributed between the two types of enzyme. (2) Microsomal ($Na^+ + K^+$)ATPase contains four large peptides per molecule, with two P_i -supported ouabain-binding sites, one nucleotide-binding site and one vanadate-binding site. The fact that protein with one vanadate-binding site has two vanadate-supported ouabain-binding sites (Fig. 2a and Table 1) makes the choice of an ATPase molecule with four large peptides obvious at this point. However, in microsomes the nucleotide-binding sites, the vanadate-binding sites and the ATP-supported ouabain-binding sites all seem to be distributed among two populations of identical size (Table 1). It therefore becomes necessary once again to make a choice between two possibilities. (1) In microsomes there are identical amounts of two different enzymes, each with one nucleotide-binding site, one vanadate-binding site and two P_i -supported ouabain-binding sites per molecule. (2) Microsomal ($Na^+ + K^+$)ATPase has four P_i -supported ouabain-binding sites, two nucleotide-binding sites and two vanadate-binding sites per molecule.

A choice can be made on the basis of the following arguments. Treatment of microsomes with SDS converts the curved binding isotherms of Fig. 2a into straight lines (Fig. 2b). This suggests that the inhomogeneity of the microsomal site populations was a consequence of interactions between pairs of inherently identical sites (for example, negative cooperativity)^{24,25}, and that treatment with SDS has disrupted the interaction.

We will disregard the possibility that it is due to chance alone that in microsomes there are equal amounts of two types of nucleotide-binding site—vanadate-binding sites and ATP-

supported ouabain-binding site—and exactly twice as many P_i -supported ouabain-binding sites and vanadate-supported ouabain-binding sites; and that SDS treatment exactly doubles the number of the former sites and quadruples the *p*-nitrophenyl phosphatase activity. Thus, for example, we deem it unlikely that one can reproducibly prepare microsomes containing appropriate proportions of inside-out, right-side-out and open vesicles with peculiar permeabilities. We must therefore choose the second possibility mentioned above, and conclude that native ($Na^+ + K^+$)ATPase as found in pig kidney microsomal preparations from the outer medulla seems to be a molecule containing eight large, catalytic peptides with four

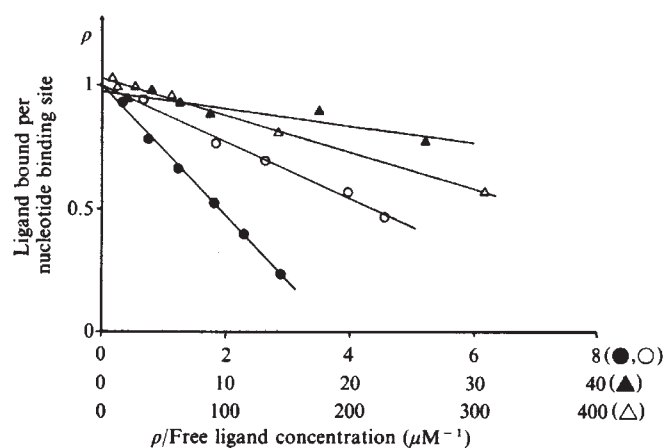


Fig. 1 Scatchard plots of data of equilibrium binding of ^{14}C -ATP (○), ^{14}C -ADP (●), ^{48}V -vanadate (▲) and of 3H -ouabain (as supported by P_i) (Δ) to a purified preparation of ($Na^+ + K^+$)ATPase from pig kidney outer medulla. The intercepts on the ordinate, calculated by linear regression analysis, give a measure of the total ligand capacities. The numerical value of each slope is equal to the dissociation constant of the ligand in question. The enzyme was prepared as described by Jørgensen¹⁵, except that ATP was omitted from the SDS activation medium. ($Na^+ + K^+$)ATPase activity (assayed according to Nørby¹⁶): 12.7 μ mol substrate split per min per mg protein. K^+ -dependent *p*-nitrophenyl phosphatase activity (assayed according to Skou¹⁷): 2.3 μ mol substrate split per min per mg protein. Nucleotide binding was measured by the method of Nørby and Jensen¹⁸. P_i -supported ouabain binding was carried out according to Hansen¹⁹ in the presence of 3 mM Mg^{2+} , 3 mM P_i and 40 mM Tris-HCl (pH 7.25, 37 °C). A similar filtration method was used for determinations of vanadate binding, after incubation of enzyme with varying concentrations of ^{48}V -vanadate for 60 min at 25 °C with 5 mM Mg^{2+} , 10 mM K^+ and 40 mM Tris-HCl (pH 7.25). ^{48}V -vanadate was from the Radiochemical Centre, Amersham and was diluted with orthovanadate obtained by dissolving $NaVO_3$ in NaOH.

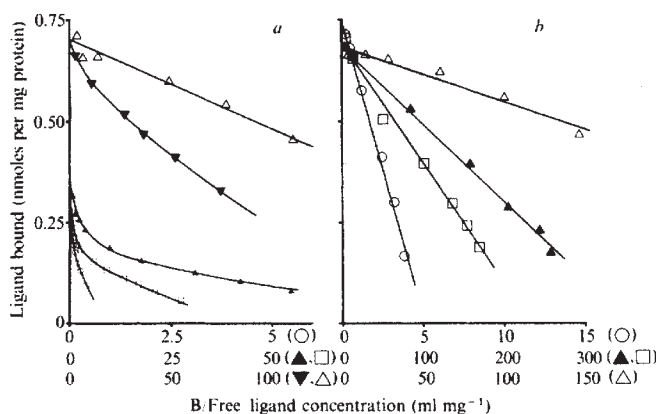


Fig. 2 Scatchard plots of data of equilibrium binding of ^{14}C -ATP ($\circ$), ^{48}V -vanadate ($\blacktriangle$), ^3H -ouabain as supported by P_i ($\triangle$), by vanadate ($\blacktriangledown$) and by ATP ($\square$) to pig kidney microsomal ($\text{Na}^+ + \text{K}^+$)ATPase prepared according to the first steps of Jørgensen's procedure¹⁵. Total ligand binding capacities can be obtained from intercepts of the curved plots with the ordinate. Non-linear regression as described in the text was used for an analysis of these curves. For the straight line plots, see text to Fig. 1. *a*, Microsomes before SDS treatment. ($\text{Na}^+ + \text{K}^+$)ATPase activity: 1.18 μmol ATP split per min per mg protein. K^+ - p -nitrophenyl phosphatase activity: 0.31 μmol substrate split per min per mg protein. *b*, Same microsomal preparation after SDS treatment. Microsomes suspended at a concentration of about 4 mg protein ml^{-1} in 250 mM sucrose, 30 mM imidazole and 0.06 mM EDTA (pH 7.2, 20°C), were treated for 20 h at room temperature with 0.06% SDS (stable, maximal K^+ - p -nitrophenyl phosphatase activity was obtained in these conditions). ($\text{Na}^+ + \text{K}^+$)ATPase activity: 6.30 μmol ATP split per min per mg protein. K^+ - p -nitrophenyl phosphatase activity: 1.19 μmol substrate split per min per mg protein. Binding of ligands was carried out in the presence of the added SDS. ATP-supported ouabain binding took place in the presence of 3 mM Mg^{2+} , 120 mM Na^+ , 3 mM ATP and 40 mM Tris-HCl (pH 7.25, 37°C). Vanadate-supported ouabain binding took place in the presence of 3 mM Mg^{2+} , 40 mM Tris-HCl and 10 μM vanadate²⁰. Other procedures were those listed in Fig. 1.

ouabain-binding sites and four nucleotide-binding sites. Of the latter, only two are available for binding substrate at any one time, that is, there is total negative cooperativity between two pairs of such sites. The nucleotide-binding sites and ouabain-binding sites would be distributed most reasonably on four identical subunits containing two large peptides each.

The result of treatment of microsomes with SDS could be explained as a relaxation of two different and coexistent interactions: the total negative cooperativity between pairs of sites and another, less overwhelming, interaction within pairs of sites. The conversion of one molecule of enzyme into four more-or-less independent subunits would be reasonable grounds for the quadrupling of p -nitrophenyl phosphatase activity that accompanies SDS treatment.

If the effects of SDS described in the present report are not due to a trivial artefact, and if they can be duplicated (by whatever physiological mechanism) *in vivo*, the proposed changes in interaction between four functional subunits of ($\text{Na}^+ + \text{K}^+$)ATPase would allow for a wide range in the regulation of the activity of the enzyme and, therefore, of the sodium pump.

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Semi-synthesis of human insulin by trypsin-catalysed replacement of Ala-B30 by Thr in porcine insulin

HUMAN insulin differs from porcine insulin by a single amino acid (Thr instead of Ala) at the C-terminal residue of the B-chain. Inouye *et al.*¹ have established a procedure for the semi-synthesis of human insulin, in which trypsin is used as a catalyst for the coupling of desoctapeptide-(B23–B30)-insulin (DOI) with a synthetic octapeptide corresponding to positions B23–B30 of human insulin. DOI was prepared by tryptic digestion of porcine insulin. The enzymatic method has many advantages, in particular, better yields and simple operation, over the chemical methods originally used by Ruttenberg² and Obermeier and Geiger³. Here we report a more simple and economical method for the semi-synthesis, in which trypsin can catalyse the coupling between desalanine-B30-insulin (DAI) and Thr-OBu^t to generate human insulin in good yield.

DAI was obtained by digestion of porcine insulin with carboxypeptidase A ($E/S = 1/100$, in weight) for 8 h at 25°C in the presence of 0.1 M NH_4HCO_3 (pH 8.3)⁴. Trypsin-catalysed coupling of DAI and Thr-OBu^t was determined quantitatively using a reverse-phase liquid chromatography (LC) system (for conditions, see Fig. 1), in which the ratio of [Thr-OBu^t-B30]-insulin produced to the remaining DAI was determined from their peak areas and intensity factors. A preliminary experiment indicated that the coupling occurred with a large excess of the amine component (Thr-OBu^t) in the presence of high concentrations of organic co-solvents. The significance of these co-solvents in coupling has been described previously^{1,5,6}. To our surprise, there was no splitting of the Arg(B22)–Gly(B23) bond in DAI or the product; this was expected to be a problem, and has previously been thought to occur through the hydrolytic activity of trypsin.

Preparative-scale coupling was carried out as follows. 0.7 ml of 0.5 M Tris buffer containing trypsin (recrystallised three times; 4.1 mg, 0.1 mM), final pH 6.5, was added to a 1.05 ml solution of DAI (100 mg, 10 mM) and Thr-OBu^t (205 mg, 0.5 M) in a mixture of dimethylformamide and ethanol (1/1 by volume), and the reaction mixture was kept at 37°C for 20 h. LC showed that 73% of the DAI had been converted into the product. The reaction mixture was applied to a column (3.2 × 41 cm) of Sephadex G-50 (super fine) and eluted with 0.5 M

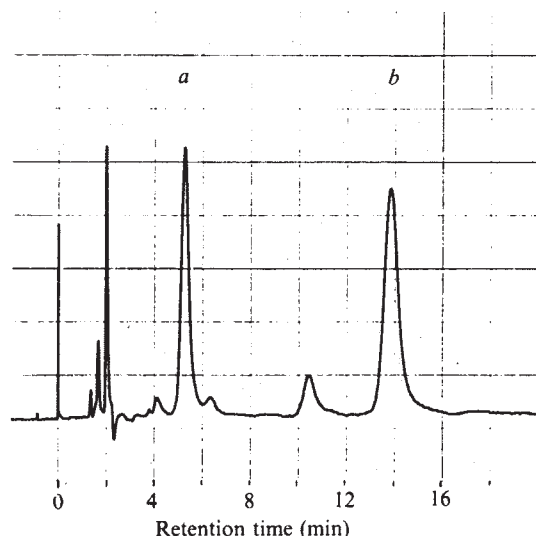


Fig. 1 Liquid chromatogram of porcine DAI (a) and [Thr-OBu^t-B30]-porcine insulin (b): column, Nucleosil 5 C₁₈, 0.4 × 20 cm; eluant, 30.5% CH₃CN in 5 mM phosphate buffer (pH 3.0) containing 5 mM n-BuSO₃Na and 50 mM Na₂SO₄; detection at 220 nm.

acetic acid. Three fractions corresponding to trypsin, insulin or its derivatives, and Thr-OBu^t were successively eluted. The fraction corresponding to insulin or its derivatives was lyophilised (89 mg), and then applied to a column of DEAE-Sephadex A-25 (2 × 20 cm) which had been equilibrated with 0.01 M Tris buffer (pH 7.4) and 7 M urea. A linear Na⁺ gradient (to 0.3 M NaCl) was begun at 4 °C according to the method of Bromer and Chance⁷. The first peak (35 mg) corresponded to [Thr-OBu^t-B30]-insulin and the second one (27 mg) to DAI and/or intact insulin (Figs 1, 2).

[Thr-OBu^t-B30]-insulin was treated for deprotection with trifluoroacetic acid in the presence of anisole. The yield was 41% (32 mg) based on the assumption that the purity of DAI used as the starting material was 78%, which had been deduced from its amino acid composition. This semi-synthetic human

insulin was homogeneous according to LC¹ and polyacrylamide gel electrophoresis (Fig. 2). Amino acid analysis (theoretical values in parentheses) gave: Lys 1.00 (1), His 1.89 (2), Arg 0.87 (1), Asp 3.32 (3), Thr 3.16 (3), Ser 2.99 (3), Glu 7.35 (7), Pro 1.29 (1), Gly 4.39 (4), Ala 1.26 (1), CySO₃H 5.38 (6), Val 3.93 (4), Ile 1.61 (2), Leu 6.51 (6), Tyr 3.68 (4), and Phe 3.19 (3). In the bioassay for hypoglycaemic activity, no significant difference was observed between the semi-synthetic human insulin and porcine insulin.

A similar preparative-scale experiment was carried out using DAI (5 mM) prepared from bovine insulin in the above conditions except that the enzyme concentration was 1 mM. LC showed that 80% of DAI had been converted into the product. The product was isolated by the above method, and pure semi-synthetic [Thr-B30]-bovine insulin was obtained in 60% yield.

The present study indicates that an enzymatic coupling system is a useful means of semi-synthesis of biologically active products, with either one or both component(s) starting from natural materials of very large molecular weight. Unlike conditions for chemical methods, for enzymatic methods functional groups in the starting materials may not need to be blocked. We must also stress that the hydrolytic activity of proteases used as a coupler is minimal in conditions suitable for synthesis, that is, in the presence of high concentrations of organic solvent and the amine or carboxyl component to be coupled.

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Fig. 2 Polyacrylamide gel electrophoresis of insulins (pH 8.0, 10% gel): 1, porcine insulin; 2, porcine DAI; 3, [Thr-OBu^t-B30]-porcine insulin; 4, semi-synthetic human insulin.

Chlorophyllin–apomyoglobin complexes

CHLOROPHYLL (Chl), *in vivo* as well as in protein complexes purified from photosynthetic organisms, absorbs light at longer wavelengths than does Chl as an isolated molecule in an organic solvent¹. These bathochromic absorbance shifts are substantial, up to 40 nm (800 cm⁻¹) for Chl *a* in green plants and 100 nm (1,400 cm⁻¹) for bacteriochlorophyll (Bchl) *a* in photosynthetic bacteria. An important question is whether these shifts are caused largely by Chl–Chl interactions or by interactions between Chl and the proteins to which all Chl is bound *in vivo*^{2–4}. This has been difficult to answer because no native Chl–protein complex yet found contains only one pigment molecule. We report here, substituting the water-soluble Chl derivative, chlorophyllin (Chln) *a*, for haem in myoglobin, and irradiating the resultant complex with red light, the first model Chl–protein complex that verifiably contains a single pigment molecule and that also exhibits a 1,000 cm⁻¹ bathochromic shift in absorbance.

For protein-free model Chl dimers in which bathochromic absorbance shifts are also observed, explanations of the shift energy have been based in part on the resonance (exciton) interaction between the electronic transitions of the two closely

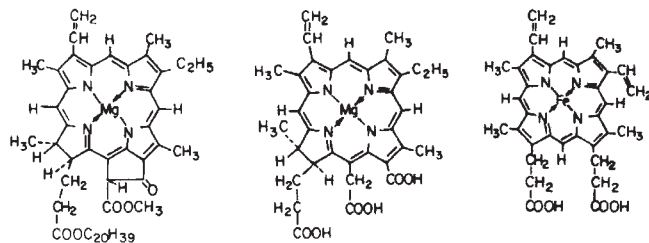


Fig. 1 Structure of (left to right): Chlorophyll *a*, Mg chlorin *e*₆ (chlorophyllin *a*), and Fe protoporphyrin IX (haem).

spaced Chl molecules⁵. A recent theoretical analysis attributes nearly half the shift energy to the exciton interaction⁶. But for two different Bchl-protein complexes about whose structure something is known^{7,8}, exciton interactions account in one case⁷ for only a quarter of the shift⁹ and in the other^{8,10} for none of it. In choosing myoglobin as our model protein, we were guided by specific findings on these two complexes. The Bchl *a*-protein from *Prosthecochloris aestuarii* is the only native complex whose structure has been determined by X-ray crystallography⁷. The Mg atoms of all 21 of its Bchl molecules are 5-coordinate; for 5/7 of the Bchls the fifth ligand of the Mg is histidine¹¹. The other is a 22,000 molecular weight purple bacterial complex (from *Rhodospseudomonas sphaeroides* R-26) that contains only two pigment molecules⁸. In this remarkable complex, the centroid of the CD spectrum is bathochromically shifted by 1,300 cm⁻¹ (relative to Bchl *a* in ether¹²), occurring at a slightly longer wavelength than that of the absorbance peak. Theoretically, in an aggregate that contains only two chromophores, the centroid of the conservative CD band, which results from the exciton interaction, occurs at the wavelength where absorbance would be maximal in the absence of that interaction¹³. Thus, analysis of the spectra of this two-pigment complex shows that in it the exciton interaction causes a small hypsochromic shift in absorbance—the entire 1,300-cm⁻¹ shift is unexplained.

In light of these considerations, apomyoglobin is almost ideally suitable as a model protein. Myoglobin itself is a small protein, and its structure is very well known from X-ray crystallography¹⁴. Its native prosthetic group, haem, is easily removed and can be replaced by another porphyrin¹⁵. In deoxymyoglobin the haem Fe atom is 5-coordinate, the fifth ligand being histidine. Also, nonspecific porphyrin-apomyoglobin binding seems unlikely. We used Breslow's method¹⁶ to prepare apomyoglobin from sperm whale myoglobin (Sigma), and subsequently purified the apoprotein by gel filtration chromatography using Sephadex G-75.

All the porphyrins so far substituted for haem in myoglobin or haemoglobin are, like haem and unlike Chl, water soluble. For simplicity, we chose to work with Mg chlorin *e*₆, a water-soluble derivative of Chl *a* commonly called chlorophyllin *a* (refs 17, 18) (Fig. 1). Chlorophyllin is Chl-like in that it has a central Mg atom and a reduced ring IV, but haem-like in having multiple carboxylates and no ring V. The structural differences between Chl *a* and Chln *a* cause only minor changes in the visible absorption spectra. We used an established procedure^{17,19} to prepare Chln *a* from Chl *a* that was purified from spinach leaves by the method of Strain and Svec²⁰.

To prepare the complexes, Chln and apomyoglobin were mixed in 0.1 M borate buffer (pH 9.2)¹⁶. The freshly prepared mixture absorbs maximally at 644 nm, bathochromically shifted only very slightly compared with free Chln in the same buffer. (Figure 2 shows the various spectral forms of Chln-apomyoglobin mixtures.) When the mixture was irradiated with red light ($\lambda \geq 600$ nm), the 644-nm peak diminished, and a new peak appeared at 690 nm. (A similar peak has been observed for Chln bound to polyvinylpyrrolidone¹⁷. In this system, a peak at ~690 nm occurs slowly in the dark and more rapidly under red

illumination. In our system the dark production rate is too small to be measured.) In the absence of O₂, the total absorbance in the red region of the spectrum is approximately conserved during the irradiation. The rate of photoconversion is temperature dependent; in the conditions used here it required ~2 h at 22 °C, while at 5 °C it was too slow to observe conveniently. In the dark at 22 °C, the 690-nm peak disappeared in a few tens of hours, and was replaced by a peak at 646 nm. Again, total red absorbance was approximately conserved, although this dark relaxation process is O₂ independent. At 5 °C, the dark relaxation proceeded ~10 times more slowly. In contrast to the original 644-nm absorbing form, the 646-nm form is insensitive to red light in the absence of O₂. The occurrence of the relaxed form apparently limits the photoconversion yield of the 690-nm form. However, we readily achieved an absorbance ratio, A_{690}/A_{645} , of 1.1.

Freshly prepared, irradiated and irradiated-and-relaxed samples (2 ml) of Chln-apomyoglobin mixtures were chromatographed separately on 1 × 50 cm Sephadex G-75 columns at 5 °C. In each case, protein and pigment co-eluted at a volume corresponding to the molecular weight of myoglobin

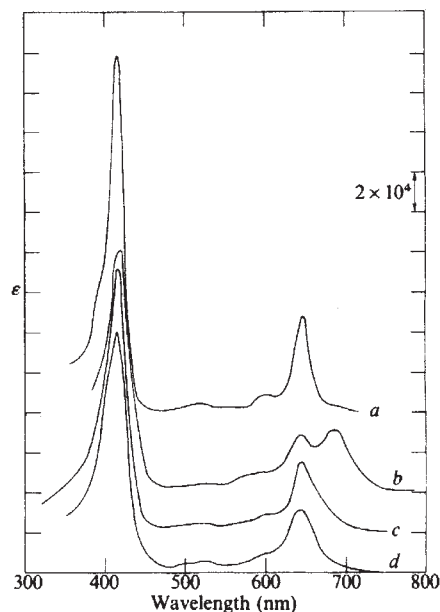


Fig. 2 Absorption spectra of: a, irradiated and relaxed; b, irradiated; c, unirradiated chlorophyllin-apomyoglobin complexes; and d, uncomplexed chlorophyllin *a*. The extinction coefficients for the protein complexes are expressed per mol apomyoglobin, and those for the uncomplexed chlorophyllin are expressed per mol pigment; the spectrum of the latter is scaled by Broyde's extinction coefficients¹⁹. All spectra have essentially zero absorbance at 800 nm. Irradiation is performed with a 75 W Xe lamp and a Corning 2-63 filter. Relaxed material remained in the dark at 22 °C for 7 days. All complexes are chromatographed on Sephadex G-75 and have 1:1 stoichiometry of pigment to protein.

(~18,000 MW), demonstrating that a complex was formed. Uncomplexed pigment eluted in much later fractions. Protein concentration was determined in the fractions by Lowry's procedure²¹, using an equimolar mixture of Chln and apomyoglobin as a standard (pigment correction, 15%). Pigment was determined spectrophotometrically for the protein complex in aqueous buffer and also in 7:2 acetone/methanol solutions. The red absorbance maximum of the unirradiated complex (644-nm form) differs little in wavelength or extinction from that of Chln in buffer. The stoichiometry of this complex in ~18,000-MW fractions taken from the column is 1.0 ± 0.1 pigment molecule per protein when either 1.2:1 or 4:1 mixtures of pig-

ment/protein are loaded onto it. Because the red absorption of the complex is substantially changed on irradiation, the pigment content of both irradiated and irradiated-and-relaxed samples was estimated by assuming that the extinction coefficient at the maximum of the Soret band (~ 420 nm) is unchanged. On this basis, a stoichiometry of 1.1 ± 0.1 pigment per apomyoglobin in 18,000-MW fractions was found when either 1.2:1 or 3:1 mixtures of pigment/protein were irradiated and then chromatographed. The irradiated-and-relaxed complex has a stoichiometry of 1.2 ± 0.2 pigment per protein. The independence of the final stoichiometries from those in the initial mixtures is further substantiated by the constancy of the ratios of red band to Soret to protein tryptophan absorbance maxima in corresponding fractions of the two chromatographies. These results demonstrate the monomeric nature of the 690-nm absorbing Chln derivative that is formed as an apomyoglobin complex.

It is likely, though not proved, that other 690-nm absorbing forms of Chln, previously observed in protein-free systems and assumed to be aggregated forms of Chln^{17,22} are also monomeric. In control experiments on irradiated Chln in protein-free borate buffer, a presumably monomeric 690-nm absorbing form is observed, together with a highly aggregated form of Chln that absorbs at 735 nm (Davis and Pearlstein, unpublished results). The latter form has also been observed in other protein-free Chln systems^{17,22}, but does not appear in solutions of apomyoglobin complexes.

The 690-nm monomer remains to be chemically identified. The close similarity of the absorbance spectra of the unirradiated and the irradiated-and-relaxed apomyoglobin complexes suggests that the chemical differences between these two are slight, and places a considerable constraint on the nature of the 690-nm form. The evidence suggests that the transformation to the 690-nm form is a property of the Chln itself. Although we do not know what this property is, one possibility is that it represents deformations of the Chln macrocycle that result from changes in axial ligation of the central Mg.

In conclusion, we have produced 1:1 complexes of apomyoglobin with the water-soluble chlorophyll derivative, chlorophyllin, that exhibit three spectral forms including one that, like chlorophyll *in vivo*, absorbs light at long wavelengths.

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An inhibitor protein of nuclear protein kinases

THE cyclical phosphorylation and dephosphorylation of proteins, catalysed by protein kinases and phosphoprotein phosphatases, respectively, are important ways in which cells regulate many of their metabolic activities. Cells seem to have at least two distinct phosphorylation systems, one in the cytoplasm, the other in the nucleus. The best studied function of the cytoplasmic protein kinases is their mediation of the effects of cyclic AMP in the modulation of existing metabolic pathways, whereas the nuclear protein kinases have been implicated in the regulation of gene activation¹. This view is based on the rapid changes in nuclear protein phosphorylation that occur in response to stimuli of differentiation and cell growth²⁻⁴, and because phosphorylated proteins are heavily concentrated in the actively transcribed regions of the genome⁵. For these reasons the nuclear protein kinases have been studied extensively, yet there is very little information concerning their regulation. It is thus of considerable interest to identify the proximal effectors of nuclear protein phosphorylation. We present here evidence for the presence in rat liver nuclei of a 150,000-molecular weight, heat-sensitive inhibitor protein that is specific for the nuclear protein kinases, being without effect on the cytoplasmic enzymes or their subunits. In addition to its potential importance as a negative regulator of nuclear protein kinases, the inhibitor may prove to be a valuable tool in assessing the role of nuclear protein phosphorylation in gene activation.

The nuclear protein kinases exist in multiple molecular forms that can be separated on ion-exchangers⁸⁻¹¹. Attempts to demonstrate a specificity of individual isoenzymes towards some of their natural substrates has remained inconclusive because of the difficulty of obtaining substrate-free enzyme and enzyme-free substrate preparations. We have used affinity chromatography, which achieves the selective removal of the protein kinases from their endogenous phosphate acceptors¹². Briefly, the procedure is to pass a crude nuclear extract, prepared as previously described¹², through a casein-Sepharose column. At 0.1 M NaCl the protein kinases are selectively retained on the column whereas the bulk of the proteins passes unretarded through the gel and is collected in the effluent (fraction I). The enzymes are then eluted from the column in a highly purified state with 0.6 M NaCl (fraction II). Enzyme activity in fraction II is demonstrable only on addition of casein or fraction I as phosphate acceptors. When incubated separately with [γ -³²P]ATP and magnesium neither fraction incorporated phosphate into trichloroacetic acid (TCA) precipitable material, but incubation of the combined fractions gave the same activity as the original extract¹².

The enzymes, essentially substrate free (fraction II), were resolved by chromatography on phosphocellulose into several peaks of activity demonstrable using casein as phosphate acceptor, as previously described⁶. The column fractions within three major peaks containing protein kinase activity were pooled and concentrated severalfold by pressure dialysis, then incubated with and without the endogenous substrates (fraction I) concentrated to contain 5 mg per ml of protein. The total reaction mixtures were analysed by electrophoresis in SDS-containing polyacrylamide gels. The results are shown in Fig. 1. Figure 1a depicts the proteins stained with Coomassie Brilliant Blue, Fig. 1b shows the autoradiograph of the same gel, depicting the proteins phosphorylated by each isoenzyme in the presence ('a' channels) and in the absence ('b' channels) of 100 μ g protein of fraction I. It can be seen that the proteins contaminating the protein kinases purified by affinity chromatography and phosphocellulose are present in quantities too small to be stained by Coomassie Blue or to register as phosphate acceptors in the standard protein kinase filter assay that measures TCA-precipitable ³²P. Nevertheless, these contaminants sustain sufficient phosphate incorporation to become visible when analysed by

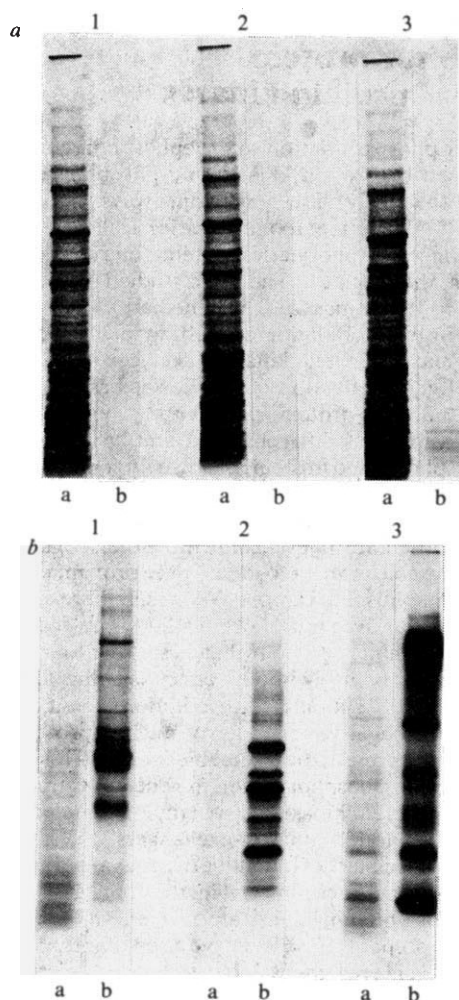


Fig. 1 Nuclear protein kinases were separated from their endogenous substrates by affinity chromatography¹² and separated into individual isoenzymes on phosphocellulose as previously described⁶. The active fractions within the three major peaks were pooled and concentrated by pressure dialysis. They were incubated for 20 min at 30 °C in 30 μ l containing 3 μ Ci [γ -³²P]ATP and 15 nmol MgCl₂ in the presence ('a' channels) and in the absence ('b' channels) of 100 μ g of enzyme-free concentrated fraction I. The reaction was stopped by addition of 3 μ l 10% SDS and the entire reaction mixture was subjected to electrophoresis in SDS-containing polyacrylamide 7–15% gradient gels. The gels were stained with Coomassie Brilliant Blue to render the proteins visible (a), then dried and exposed for 16 h to NS 5T Kodak X-ray film (b). The twin-channels labelled 1, 2 and 3 refer to different isoenzymes.

autoradiography on gels after a 16-h exposure. It is clear that the latter detection method is considerably more sensitive than the filter assay. In the presence of 100 μ g of protein in fraction I, however, phosphate incorporation is nearly abolished, whereas fraction I proteins supported significant phosphate incorporation when present at concentrations of 5–10 μ g per assay. These results suggested that the fraction containing endogenous substrates also contained an inhibitor of protein kinase which, on concentration of crude, but enzyme-free, extracts severely inhibits phosphate incorporation into the natural substrates.

This preparation inhibited not only the phosphorylation of nuclear proteins but also that of casein. This permitted us to quantify the amounts of inhibitor present in nuclear extracts in the presence of saturating amounts of casein (1 mg per ml), thus eliminating the effects of varying amounts of endogenous substrates in the assay. Studies to characterise the inhibitor therefore measured the inhibition of casein phosphorylation. Figure 2 shows the inverse proportionality of remaining protein kinase activity with increasing amounts of inhibitor added to a constant amount (0.1 unit) of protein kinase purified by affinity chromatography¹². One unit of inhibitor is defined as the amount giving

50% inhibition of 0.1–0.2 units of nuclear protein kinase in the standard assay¹².

Fraction I was adsorbed to a DEAE-cellulose column at 6 mg per ml of packed column volume in 50 mM Tris-HCl, pH 6.8, containing 0.1 M NaCl and 0.05% Nonidet P-40 (buffer I). Inhibitory activity eluted as a sharp peak between 0.3 and 0.35 M NaCl when the column was developed with a linear gradient in NaCl of 0.1–0.5 M in 50 mM Tris-HCl, pH 6.8, and 0.05% Nonidet P-40. The active fractions were pooled, concentrated by pressure dialysis and loaded onto a 0.6 $\times$ 50 cm column of Biogel P-150 (minus 400 wet mesh) equilibrated and developed in 50 mM Tris-HCl, pH 6.8, containing 0.5 M NaCl to minimise the possibility of molecular aggregation. The inhibitor emerged as a single, slightly skewed peak with an R_F of 0.9 relative to dextran blue, indicating an apparent molecular weight of 150,000. Inhibitory activity was destroyed on incubation at 60 °C for 10 min at pH 7 or at 37 °C for 30 min at pH 4. Incubation of the inhibitor with pancreatic RNase, DNase I or phospholipase A at 37 °C for 60 min had no effect, whereas incubation with trypsin or chymotrypsin rapidly destroyed its activity (data not shown).

Aliquots of an inhibitor preparation purified through the DEAE-cellulose step, containing 30–50 μ g protein, induced 30–40% inhibition of each of the three major isoenzymes of nuclear protein kinase separated on phosphocellulose. However, they had no effect on the activity of a cytoplasmic protein kinase preparation (100,000g rat liver supernatant) either with casein or with histones as substrates, regardless of whether cyclic AMP was present in the assay. The results of a typical experiment are summarised in Table 1. The specificity of the inhibitor for the nuclear protein kinases ruled out the possibility that inhibition was due to some nonspecific interference with the assay, such as ATPase or protease activities in the extract, because such activities would also give an apparent inhibition of the phosphotransferase reaction catalysed by the cytoplasmic protein kinases. It did not eliminate the possibility that the inhibitor was a phosphoprotein phosphatase, as we have previously shown that the nuclear phosphoprotein phosphatases have a pronounced substrate preference for nuclear phosphoproteins over phosphoprotamine¹³. However, this possibility could also be discounted, as when nuclear phosphoproteins, prepared from livers of rats injected with ³²P 90 min before death, were incubated with the inhibitor for 30 min at 30 °C, there was no loss of phosphate from TCA-precipitable material.

We have previously shown that the nuclear protein kinases, in contrast to the cytoplasmic enzymes, are stimulated by polyamines, but not by cyclic AMP^{6,7}, and are not inhibited by the

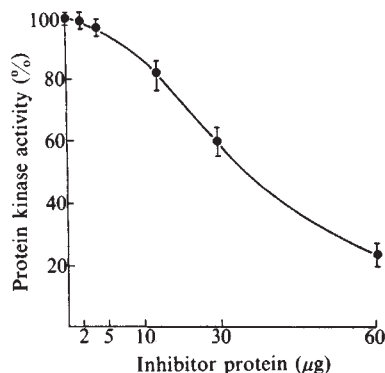


Fig. 2 Nuclear protein kinase (0.1 unit) was incubated with various amounts of inhibitor protein purified through the DEAE-cellulose step as described in the text. The inverse proportionality between remaining enzyme activity and amounts of inhibitor added is that predicted by the Michaelis-Menten relation for the reversible interaction between an enzyme and its inhibitor.

Table 1 Effect of the inhibitor protein on nuclear and cytoplasmic protein kinases

Protein kinase	Substrate	Other additions	nmol ³² P incorporated per min per mg	% Inhibition
Nuclear				
Phosphocellulose				
Peak 3	Casein	None	0.49	
	Casein	Inhibitor	0.34	31
Peak 4	Casein	None	1.32	
	Casein	Inhibitor	0.83	37
Peak 5	Casein	None	0.25	
	Casein	Inhibitor	0.15	40
Cytoplasmic				
	Casein	None	0.46	
	Casein	Inhibitor	0.46	0
	Casein	+ cyclic AMP (5×10^{-6} M)	0.48	
	Casein	+ cyclic AMP + inhibitor	0.49	0
	Histone	None	0.76	
	Histone	Inhibitor	0.74	2.5
	Histone	+ cyclic AMP (5×10^{-6} M)	3.05	
	Histone	+ cyclic AMP + inhibitor	2.97	2.5

Nuclear protein kinases were prepared and freed of endogenous substrate¹² and fractionated on phosphocellulose as previously described⁶. Cytoplasmic protein kinase was a 100,000g rat liver supernatant. Casein and histones were present at 200 µg in the 0.2-ml standard assay¹².

* Inhibitor protein (30 µg) was purified by the DEAE-cellulose step as described in the text.

cytoplasmic heat-stable inhibitor protein⁶ that specifically inhibits cyclic AMP-dependent protein kinases by interacting with their catalytic subunit¹⁴. Here we report the presence in rat liver nuclei of a heat-sensitive inhibitor of 150,000 molecular weight that behaves as an acidic protein and is specific for the nuclear enzymes. Taken together, these findings suggest that the nuclear protein kinases are subject to controls that are specific and distinct from those regulating the cytoplasmic enzymes which primarily mediate the effects of cyclic AMP.

We believe this inhibitor to represent the only negative modulator of nuclear protein kinase activity described to date. It is suggested that the inhibitor may play an important part in the regulation of nuclear protein phosphorylation. Moreover, it can be expected to be a valuable tool in assessing the possible role of nuclear protein phosphorylation in nuclear RNA metabolism and gene activation.

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A chiasma-hormonal hypothesis relating Down's syndrome and maternal age

THE incidence of the human congenital abnormality known as Down's syndrome (or mongolism) has been observed to increase with maternal age¹. The trend of sharply increased incidence beyond the age of 30 has been well documented², and various hypotheses have been formulated to explain it. These include cumulative ovum or uterine dysfunction (radiation³, chemicals⁴, disease⁵), acute effects of radiation and chemicals, hormonal effects⁶, the 'production line' hypothesis^{7,8} and changes in sexual behaviour⁹. We describe here our hypothesis that there is an interaction between the hormonally governed rate of meiosis and the timing of chiasma terminalisation. We propose that changing hormone levels during the menstrual cycle not only trigger resumption of meiosis in the ovum¹⁰, but also control the rate of meiosis through the availability of a limiting substance¹¹. As hormone levels and the length of the cycle change with advancing age of the mother, meiosis slows down, and chiasma frequencies decline^{7,12}. Meiotic chromosome bivalents are held together by their chiasmata against strong mutual repulsion during late dictyotene and diakinesis¹³, making smaller bivalents with fewer chiasmata, such as 21, the most vulnerable to premature separation during terminalisation^{14,15}; premature separation can lead to trisomy 21, by far the most frequent cytological manifestation of Down's syndrome¹⁶.

17β-Oestradiol may stimulate the production of luteinising hormone (LH) receptors in the follicle either directly through transcription^{17,18} or indirectly by increasing the number of granulosa cells surrounding the ovum¹⁹. Thus, LH receptors could accumulate during the menstrual cycle as an integral function of oestradiol concentration. At corresponding points in the cycle, LH concentration varies little²⁰, but oestradiol decreases with age^{20,21} (see Fig. 1 legend).

After meiosis resumes in the ovum at the LH surge, the rate of meiosis is assumed to be limited by a substance released in response to LH¹⁹ (for example, cyclic AMP¹⁰ or Ca²⁺ (ref. 22)). As LH concentration remains high during this part of the cycle, the intensity of the LH stimulus may be limited by the number of LH receptors rather than by LH concentration¹⁹. In this simplest case, the accumulation rate of the limiting substance is directly proportional to the number of receptors. By ignoring allosteric or other complexities of the rate-limiting reaction, the rate of meiosis can be expressed as the Michaelis-Menten function in equation (1) (Fig. 1 legend).

Estimating the minimal interval from LH peak to ovulation to be 12 h (ref. 23), we add 24 h for the duration of the LH surge²⁴ to obtain the interval from resumption of meiosis to ovulation (end of meiotic division I) in women at age 20; we can then use equation (1) to predict this interval for other ages. Bivalents become attached to spindle fibres roughly 18 h after the resumption of meiosis in women at age 20 (ref. 25), but equation (1) implies that this pre-alignment interval lengthens for older women, with an increasing chance of losing chiasmata before proper alignment. We assume that the starting times of terminalisation can be represented by a normal distribution independent of age. As a first approximation, let the frequency of premature univalents be given by the frequency of bivalents whose chiasmata terminalise before spindle attachment. Then the frequency of prematurely terminalised chiasmata is expressed by equation (2) (Fig. 1 legend). Note that if terminalisation of individual bivalents is rapid relative to the time scale of meiosis, as seems likely, the exact number and positions of chiasmata become unimportant.

Assuming that unaligned univalents 21 successfully attach to the spindle but segregate independently at anaphase I (ref. 8), allowing for a partial fetal viability (we use 0.235 (ref. 26)) and multiplying by 1,000, we obtain the incidence per 1,000 births of trisomy 21 as a function of maternal age in equation (3) (Fig. 1

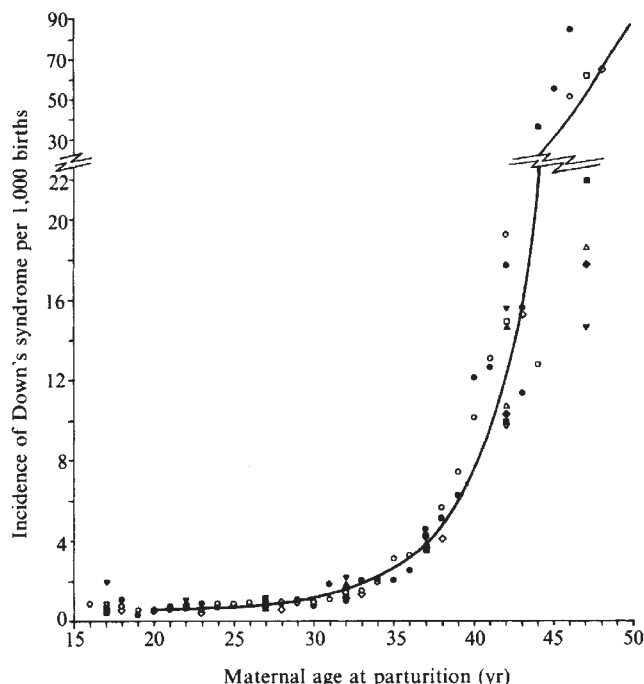


Fig. 1 Incidence of Down's syndrome per 1,000 births versus maternal age at parturition. Data from eight of the studies cited are for 5-yr age intervals; data from the last two studies are for 1-yr intervals. The curve illustrates one reasonable fit to these data of the model developed here, for which the two fitted parameters are $\sigma = 7.09$ and $K_m/k_1k_0 = 7,000 \text{ h}^2$. This result assumes a reverse sigmoid decline beyond age 20 in peak oestradiol concentration ($c(a) = 1,176/(a^2 - 40a + 1,576)$) and a linear peak shift ($p(a) = 603 - 8.53a$, where p is time in hours to the oestradiol peak from the beginning of the cycle) with age a in years. The general forms and parameters of these two functions are suggested by data in Sherman and Korenman²⁰. Key equations from the derivation outlined in the text are:

$$\rho(a, t) = \frac{\rho_{\max} M(a, t)}{K_m + M(a, t)} = \frac{\rho_{\max} c(a) \int_0^t E(u) du ds}{K_m/k_1k_0 + c(a) \int_0^t E(u) du ds} \quad (1)$$

where ρ is the rate of meiosis at time t during the cycle of a woman of age a , h^{-1} ; $\rho_{\max}$ is the maximal rate of meiosis ('limiting' substrate present in excess), h^{-1} ; M is the concentration of the rate-limiting metabolite, ng l^{-1} ; a is maternal age, yr; t is time during the menstrual cycle, h; K_m is the Michaelis constant, ng l^{-1} ; c expresses the decline in oestradiol concentration with age a , normalised such that $c(20) = 1$; t_0 is an initialisation time, taken here to be the beginning of the cycle; E is the oestradiol concentration during the cycle, ng l^{-1} ; k_1 is the rate constant for metabolite accumulation, h^{-1} ; and k_0 is the coefficient for the accumulation of LH receptors, $\text{ng l}^{-1} \text{h}^{-1}$.

$$F(f) = \int_0^f P(r) \phi(r) dr$$

where

$$P(r) = \begin{cases} 0 & \text{if } f \leq r \\ \alpha(f-r) & \text{if } f > r \geq f-1/\alpha \\ 1 & \text{if } f-1/\alpha > r \end{cases} \quad (2)$$

and

$$\phi(r) = \exp[-(r-\tau)^2/2\sigma^2]/2\pi\sigma$$

and where F is the frequency of unaligned univalents; f is the pre-alignment fraction of the interval between resumption of meiosis and ovulation; r is the duration of this entire interval, h; $P(r)$ is the probability of terminalisation by time r (h) after the resumption of meiosis; α is the terminalisation rate, h^{-1} ; ϕ is the frequency distribution of first terminalisation times, of which τ is the mean and σ is the standard deviation, h.

$$I(a) = 1,000vF(f(a))/(2+v) \quad (3)$$

where I is the incidence per 1,000 births of trisomy 21, and v is the fraction of 21-trisomic zygotes surviving to birth divided by the fraction of all other zygotes surviving to birth. ♦, ref. 33; △, ref. 34; ■, ref. 35; ▼, ref. 36; □, ref. 37; ▲, ref. 38; ▽, ref. 39; ◇, ref. 40; ○, ref. 41; ●, ref. 42 (corrected for sparsity of data).

legend). The curve in Fig. 1 illustrates a reasonable fit to the data of equation (3) found by appropriately adjusting the two parameters σ and K_m/k_1k_0 .

The curve in Fig. 1 accelerates sharply in response to both the $i(a)$ function and the leading tail of the cumulative normal function $\int \phi(r) dr$. By the analysis presented here, neither the hormonal (i) nor the chiasma (ϕ) effects alone can bend the incidence curve sharply enough to fit the data, but taken together, as in equation (2), they can provide sufficient acceleration.

The chiasma-hormonal hypothesis proposed here yields some testable (but as yet untested) predictions: (1) The interval during the cycle between the LH peak and ovulation increases with maternal age (see refs 27, 28). (2) The incidence of trisomies will respond to experimental manipulation of intrafollicular hormone levels (see refs 29, 30). (3) Chiasma frequencies in the bivalents 21 of human females decrease with age during post-arrest dictyotene and diakinesis (see refs 7, 11 in mice).

Our hypothesis clearly emphasises the need for further experimental studies that link hormonal signals with cytological events, and for further information on the dynamics and significance of chiasmata before and during segregation. Thorough documentation of intrafollicular hormone levels during a cycle would be particularly useful, avoiding the necessity for extrapolating (as here) from systemic levels to follicular levels.

We have not formally considered the incidence trend below a maternal age of 20 yr in the model presented here. We suspect that the typically higher incidence of Down's syndrome in children of mothers younger than 20 (see Fig. 1) results from a higher variability of hormone levels among cycles as the complex neuro-endocrine feedback controls are still being established³¹; none of the other mechanisms assumed to operate in meiotic division I could so easily account for this higher incidence for very young mothers. The behavioural mechanism is not necessarily inconsistent with this pattern below age 20, but the finding that the majority of maternal meiotic segregation errors occur during division I (ref. 32) seems to preclude a significant behavioural effect.

Many other specific models, perhaps focusing on other hormones (follicle-stimulating hormone, progesterone) or other hormone interactions, could probably fit the data of Fig. 1 equally well. The parsimonious LH-oestradiol interpretation is primarily intended to illustrate the essential implications of the general chiasma-hormonal mechanism.

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Specific DNA methylation sites in the vicinity of the chicken β -globin genes

METHYLATION of cytosine is the only post-synthetic modification so far detected in the DNA of higher eukaryotes and thus has been made the basis of several proposed mechanisms of gene activity and cellular differentiation^{1–3}. All evidence indicates, however, that the overall content of 5-methylcytosine in DNA does not vary significantly between different tissues of the same organism and does not change throughout at least some steps of differentiation^{4–6}. As 5-methylcytosine occurs predominantly in the dinucleotide sequence CpG (ref. 7) and as a number of bacterial restriction endonucleases can distinguish whether this sequence is methylated or unmethylated, it is now possible to investigate DNA methylation patterns in the vicinity of single genes⁸. In this report, we use this technique to provide a correlation between DNA methylation and the activity of the chicken β -globin genes.

The restriction endonuclease *HpaII* cleaves the DNA sequence CCGG (ref. 9) but does not cleave the methylated sequence C(MeC)GG (ref. 10). A second restriction endonuclease, *MspI*, is able to cleave this same DNA sequence whether it is methylated or not¹¹. Thus DNA from any cell or tissue type can be digested with either *HpaII* or *MspI*, the cleaved DNA electrophoresed on an agarose gel, transferred to a nitrocellulose filter¹² and hybridised to a gene-specific probe. Any bands seen in the autoradiogram which differ between the two enzyme digests can reasonably be ascribed to differences in methylation of CCGG sites in the vicinity of the specific gene. This kind of experiment, used here to examine the chicken β -globin genes, has recently been used to investigate a single methylation site in the large intervening sequence of the adult rabbit β -globin gene¹³.

Figure 1 shows the autoradiogram from an experiment with chicken DNA, using the plasmid pHb1001 as a ³²P-labelled hybridisation probe (pHb1001 is a cDNA clone of the adult chicken β -globin gene¹⁴, provided by Dr W. Salser). Figure 1a

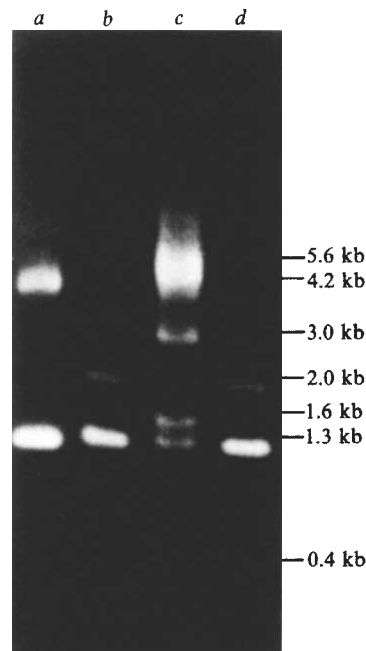


Fig. 1 DNA was isolated from chicken tissues by extensive digestion with proteinase K (Merck) in the presence of 0.2% SDS with or without previous isolation of nuclei. This was followed by at least three extractions with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), three extractions with two volumes of chloroform-isoamyl-alcohol (24:1), and three ethanol precipitations. In a typical digestion protocol, 200 units of restriction enzyme were added to 100 μ g DNA in a final buffer of 6 mM KCl, 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol and 100 μ g ml⁻¹ autoclaved gelatin. (The enzyme *MspI* was obtained from New England Biolabs. The enzyme *HpaII* was obtained from either New England Biolabs, Bethesda Research Laboratories, or Boehringer-Mannheim; all gave identical results. It was verified that, in the digestion conditions used, both *HpaII* and *MspI* gave identical cleavage patterns with the plasmid pBR322.) The reaction was incubated at 37 °C for 2 h, a further 200 units of enzyme were added and incubation was continued for an additional 2 h at 37 °C. Identical results were obtained if this second incubation was continued for 10–15 h. The completion of digestion was monitored by adding a small amount of bacteriophage λ DNA to an aliquot of either the first or the second incubation mixture and verifying by electrophoresis that the required cleavage products were present. After digestion, the DNA was reprecipitated by repeating the proteinase K digestion and organic extraction steps described above, and 25–50 μ g electrophoresed on each slot of a 1% agarose slab, 6 mm thick, for 2 h at 200 V. DNA was then transferred to Schleicher and Schuell nitrocellulose filters by the method of Southern¹², the filters hybridised to ³²P-labelled nick-translated pHb1001 for 24 h at 70 °C in 6 $\times$ SSC, washed in 3 $\times$ SSC, and finally washed for 30 min in 0.1 $\times$ SSC. Filters were exposed to Kodak XR5 X-ray film for 24–96 h at –80 °C, using two Dupont Cronex intensifying screens. A typical autoradiogram is shown above and described in more detail in the text. *a*, Adult erythrocyte DNA digested with *HpaII*; *b*, adult erythrocyte DNA digested with *MspI*; *c*, oviduct DNA digested with *HpaII*; *d*, oviduct DNA digested with *MspI*. Band sizes were calibrated relative to a *HindIII* digest of λ and a *HaeIII* digest of Φ X174 DNA, run in an adjacent gel slot, and are probably accurate to 5%. Identical *HpaII* digestion patterns were obtained with DNA from erythrocytes or reticulocytes isolated from several individual adult roosters and chickens, of both White Rock and Leghorn breeds. *HpaII* digestion of a mixture of erythrocyte and oviduct DNA yielded a pattern which was the expected sum of the patterns of slots *a* and *c* above. A digest of the same DNA with a mixture of *HpaII* and *MspI* yielded only the *MspI* digestion pattern. The *MspI* digestion pattern of all DNA samples investigated was identical to that in slots *b* and *d* above. Digestion with several restriction endonucleases which do not have CG as part of their recognition site gave the same digestion patterns in different tissues (see also ref. 16).

represents the *HpaII* digest of adult chicken erythrocyte DNA and shows bands at 4.2 and 1.3 kilobase pairs. These fragment sizes should correspond to distances between unmethylated CCGG sites in the DNA regions containing β -globin genes. Figure 1b represents the *MspI* digest of the same adult erythrocyte DNA and shows bands at 2.0 and 1.3 kilobases, as well as a faint but reproducible band at 0.4 kilobases. These fragment sizes should correspond to distances between both methylated and unmethylated CCGG sites in the β -globin gene DNA. The 1.3-kilobase fragment is of similar intensity in both digests.

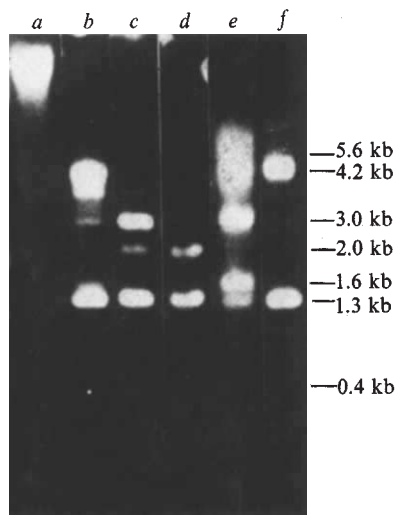


Fig. 2 *a-d*, Partial digestion of adult erythrocyte DNA with the restriction endonuclease *MspI*; *a*, no enzyme control; *b*, 2 units; *c*, 12 units; *d*, 100 units of enzyme. All incubations were for 2 h at 37 °C with 50 µg DNA and using the buffer conditions described in Fig. 1. *e*, Complete *HpaII* digest of erythrocyte DNA from 5-d-old embryos. For comparison, slot *f* is a complete *HpaII* digest of adult erythrocyte DNA.

However, the *HpaII* digest (*a*) lacks both the 0.4 and 2.0 kilobase fragments of the *MspI* digest as well as any other bands which could correspond to a combination of the *MspI* bands. This suggests that the CCGG sites at the ends of the 1.3-kilobase fragment are completely (>90%) unmethylated in adult chicken erythrocyte DNA and furthermore, that at least two other specific sites are completely methylated. DNA isolated from the circulating reticulocytes of phenylhydrazine-induced anaemic adult chickens gives digestion patterns which are essentially identical to those of adult erythrocyte DNA (data not shown). These results are in contrast to those for the rabbit β -globin gene¹³, where in both marrow and spleen cells of anaemic rabbits, only partial methylation of a site in the large intervening sequence was observed; this difference could be due to the different positions of the cleavage sites within the gene sequence or, as the authors have pointed out¹³, due to a heterogeneous cell population.

Figure 1c shows the *HpaII* digest of oviduct DNA. The observed band pattern is not only different from the *MspI* digest of the same DNA (Fig. 1d) but also different from the *HpaII* digest of erythrocyte DNA (Fig. 1a). There are now prominent bands at 4.2, 3.0, 1.6 and 1.3 kilobases (as well as several weaker but poorly resolved bands at higher molecular weight). As Fig. 2b and c show, all these bands appear as partial digestion products of adult erythrocyte DNA by the enzyme *MspI*. This suggests that, in the oviduct, CCGG sequences in the vicinity of the β -globin genes are only partially methylated. Nevertheless, the absence of the 2.0-kilobase band suggests that at least one such sequence is completely methylated. As shown in Fig. 2e, DNA isolated from the circulating red blood cells of 5-d-old embryos (in which the adult β -globin gene is not being expressed^{14,15}) or from adult brain (data not shown) gives digestion patterns essentially identical to that of oviduct DNA.

The legend to Fig. 1 summarises control experiments which show that the above results are not due to partial digestions, DNA isolation methods, enzyme inhibitors, DNA sequence rearrangements or individual differences between chickens. Figure 2a also provides an important control, showing that undigested DNA is still transferred to the nitrocellulose filter for hybridisation; thus, any high molecular weight bands would have been detected.

One current difficulty in assigning the observed bands to their positions within the gene sequence arises from the fact that the adult chicken β -globin gene in pHb1001 shares considerable sequence homology with at least one of the embryonic β -globin

genes¹⁶, and both sequences can show up on the filter hybridisations. However, our preliminary results using a genomic clone (G.D.G., W. I. Wood and G. Felsenfeld, unpublished) indicate that the prominent 1.3-kilobase fragment shown in Figs 1 and 2 is derived from the adult β -globin gene sequence; moreover one end of this fragment lies within about 30 bases of the 5'-end of the coding sequence¹⁴.

It is undoubtedly premature to relate DNA methylation patterns to a specific biological activity. It does seem clear, however, that the observed tissue differences do not solely reflect different rates of cell division, as both adult and embryonic red cells have ceased division and yet their DNA shows different methylation patterns. Moreover, brain, a non-proliferating tissue, shows the same pattern as the proliferating oviduct. On the other hand, at least some correlation can be made between these methylation patterns and globin gene activity. In cells which are expressing or have expressed the adult β -globin gene (adult reticulocytes and erythrocytes) the CCGG sites near the ends of the gene sequence (that is at the ends of the 1.3-kilobase fragment) seem to be completely unmethylated. In cells which are not expressing this gene (oviduct, brain and embryonic red blood cells) these sites can be at least partially methylated. The determination of how these specific methylation events occur during cellular differentiation must await a more detailed description of their exact positions in the gene sequence. Nevertheless, we believe these results to be the first correlation between site-specific DNA methylation and eukaryotic gene expression.

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Long regions of single-stranded DNA in human cells

WHEN DNA is isolated from actively growing animal cells by a variety of standard procedures, 1-2% of the total DNA is recovered in single-stranded form¹⁻⁵. Pulse labelling experiments have established that the large majority of the single-stranded DNA is not newly synthesised^{3,5}. However, a minor part of this DNA fraction consists of short fragments of newly replicated DNA released by branch migration during isolation^{6,7}. Studies with synchronised human cells have shown that single-stranded DNA is mainly found during the period of DNA synthesis^{3,4}. In agreement with this, a specific antiserum against denatured DNA used for immunofluorescence has revealed the presence of single-stranded DNA in nuclei of a human lymphoid

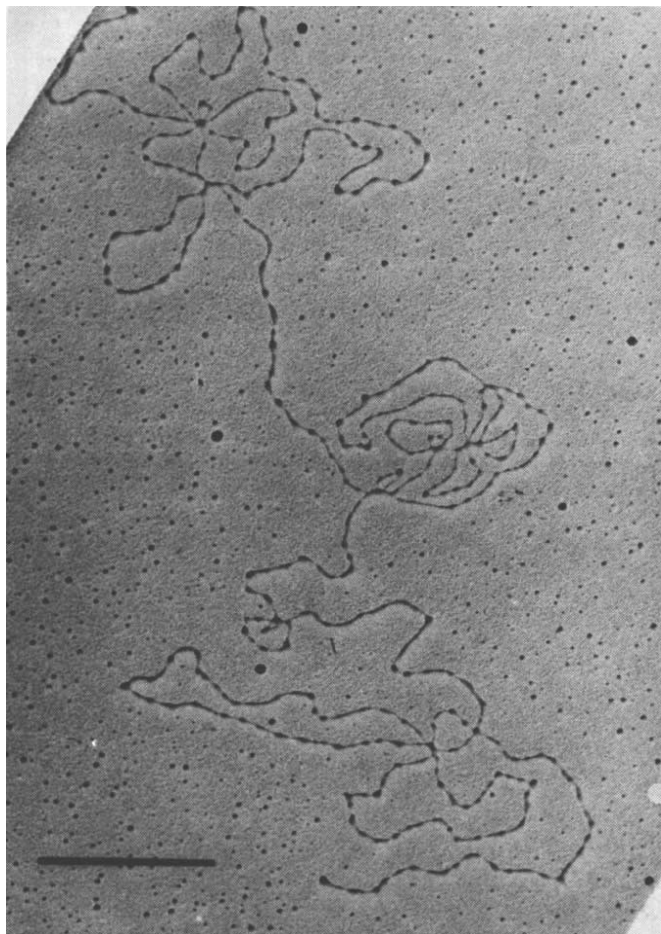


Fig. 1 Electron micrograph of a long single-stranded DNA molecule isolated from the human lymphoid cell line Molt-4. The DNA was spread in the presence of 30% formamide by the technique of Davis *et al.*¹⁵. In comparison with an internal reference, phage Φ X174 DNA, this molecule has a molecular weight of 40×10^6 . Scale bar, 1 μ m.

cell line during the S phase but not during the G1 phase⁸. DNA hybridisation measurements indicate that the single-stranded sequences belong to the non-repetitious fraction of the DNA and that at least part of this material contains sequences transcribed *in vivo*². We have developed gentle isolation methods to purify large, covalently closed circular DNA molecules from human lymphocytes transformed by the Epstein-Barr virus (EBV)⁹. These methods do not cause the conversion of linear double-stranded DNA to single-stranded forms^{5,9}. We have repeatedly observed by electron microscopy long single-stranded stretches of host DNA that initially co-purified with the guanine-cytosine-rich viral episomal DNA. This single-stranded DNA accounted for about 1% of the total cellular DNA in several different lymphoid cell lines derived from Burkitt lymphoma patients or from healthy donors, while the EBV DNA sequences in the same lines only constituted 0.02–0.1% of the total DNA. Clearly, the single-stranded sequences were mainly or exclusively of host origin. Long regions of single-stranded DNA, indistinguishable from those present in EBV-transformed cells, were also found in similar amounts in two EBV-negative human lymphoid cell lines of different origin, BJAB¹⁰ and Molt-4 (ref. 11). This report looks at these single-stranded DNA molecules, which are considerably larger than any previously isolated from animal cells.

Molt-4 cells were grown in stationary suspension culture at 37°C in RPMI-1640 medium (GIBCO) supplemented with 15% fetal bovine serum and antibiotics. High molecular weight DNA was prepared by treatment of washed cells with Sarkosyl

and Pronase, followed by CsCl density gradient centrifugation⁹. Fractions of higher density ($1.715\text{--}1.735\text{ g cm}^{-3}$) than the main band DNA (1.700 g cm^{-3}) were recovered with the aid of a closed system collection device to minimise shearing forces⁹. This material, which contained about 5% of the total cellular DNA, was further fractionated by neutral glycerol gradient centrifugation in the presence of 1 M NaCl. The conditions were as described previously⁹, except that the time of centrifugation was reduced to 2 h, polyallomer tubes were used, and the size marker (phage T4 ^{14}C -DNA) was run in a separate tube. The purpose of the latter fractionation step was to purify further large single-stranded DNA molecules, which sediment faster than double-stranded molecules of similar size¹². Different fractions of a glycerol gradient were dialysed free from glycerol, and circular single-stranded DNA from bacteriophage Φ X174 was added as an internal reference. The fractions were then examined by electron microscopy, using the formamide modification of the Kleinschmidt technique¹³.

The fastest sedimenting DNA detected had a sedimentation coefficient of 150–160S. This DNA seemed to be entirely single stranded, as judged by electron microscopy (Fig. 1). Using contour length measurements the DNA was estimated to have a molecular weight of 40×10^6 . Note that a single-stranded DNA molecule of this size would be expected to have a sedimentation coefficient of 156S in neutral 1 M NaCl (ref. 12). Furthermore, these long DNA molecules as well as the Φ X174 DNA were no longer detected after treatment with pancreatic DNase or single-strand-specific S_1 nuclease, while they were resistant to pancreatic RNase.

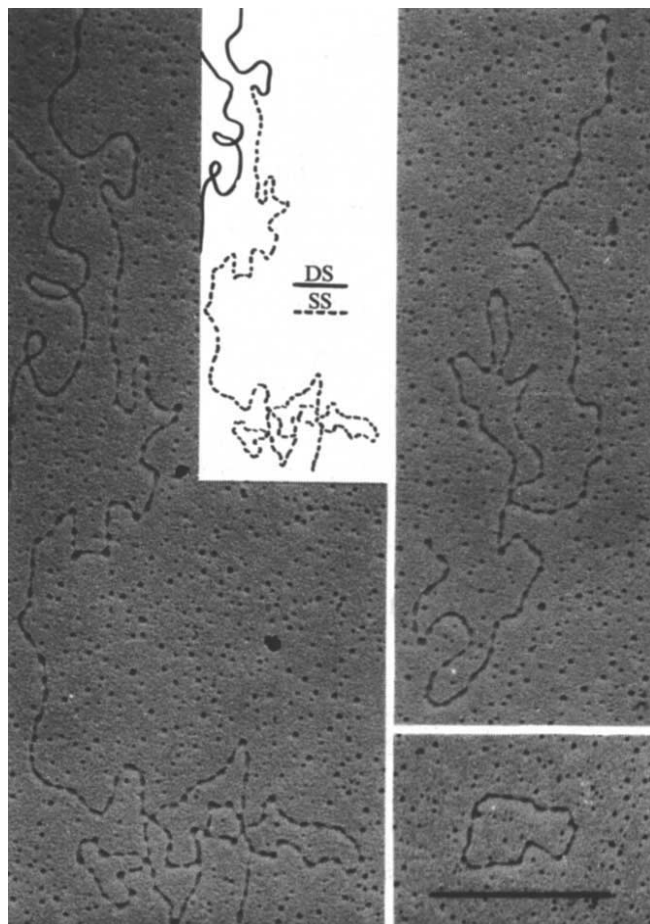


Fig. 2 Single-stranded DNA from Molt-4 cells. Left, a molecule comprising both double-stranded (DS) and single-stranded (SS) DNA. The length of the single-stranded region is 17×10^6 MW. Upper right, a well extended single-stranded DNA molecule of 14×10^6 MW. Lower right, single-stranded circular DNA from bacteriophage Φ X174 of 1.8×10^6 MW. Scale bar, 1 μ m for all pictures.

Table 1 Single-stranded DNA from cells in different growth phases

	Sample A (early S phase)	Sample B (mid S phase)	Sample C (G2 phase)
Number of double-stranded DNA molecules per field square	6.1	5.4	7.4
Number of double-stranded molecules measured	43	38	52
Average length of double-stranded DNA (μm)	38.5	43.2	32.1
Number of single-stranded DNA molecules per field square	1.2	0.9	0.6
Number of single-stranded molecules measured	29	23	16
Average length of single-stranded DNA (μm)	18.0	23.5	11.3
Proportion of DNA in single-stranded form (%)	9.2	9.1	2.9

DNA preparations were spread in the presence of 30% formamide¹³. Bacteriophage PM2 DNA in open circular form (MW 6.4×10^6) was used as size reference. A length correction (based on parallel measurements of double-stranded and single-stranded ΦX174 DNA) was performed to correct for differences in spreading between double-stranded and single-stranded DNA.

Most of the high molecular weight DNA investigated here sedimented as a broad peak around 70S on glycerol gradients. In the region of a gradient containing molecules sedimenting at $>50\text{S}$, which presumably would be free from any newly replicated DNA fragments of low molecular weight, about 20% of the total material was found in single-stranded form (Fig. 2). The size distribution of the single-stranded DNA recovered is shown in Fig. 3. It seems that the single-stranded DNA is not of unique size, and that a considerable part of this material is as much as $20\text{--}40 \times 10^6$ MW. However, the long DNA chains are very shear sensitive, and could be derived from even larger single-stranded or partly single-stranded molecules, fragmented during isolation.

To investigate the alkali stability of the material, a fast-sedimenting DNA fraction (100–120S) containing a relatively large amount of single-stranded DNA was brought to pH 12.7 by the addition of NaOH, left at 20°C for 15 min, neutralised, and immediately spread for electron microscopy. A control experiment with bacteriophage λ DNA showed that double-stranded DNA was completely converted to intact single strands in these conditions. An aliquot of a 100–120S DNA fraction isolated from Molt-4 cells contained 26 DNA molecules, of which 22 were single-stranded molecules between 11 and 35 μm long, and 4 were double-stranded molecules. A similar aliquot from the alkali-denatured material contained 7–42- μm long single-stranded DNA molecules. There was no detectable decrease in the average length (about 20 μm) of the chains after exposure to alkali. These data show that the material shown in Figs 1–3 consists of long single-stranded DNA molecules rather than hydrogen-bonded aggregates of short chains.

As it has been reported that single-stranded DNA is primarily found in growing cells in S phase^{3,4,8}, it was of interest to see if the much longer single-stranded regions detected here were also mostly present during active DNA synthesis. Human lymphoid cells were therefore synchronised by the double thymidine blocking technique¹⁴. This procedure is of varying effectiveness with different lymphoid lines, but Raji cells respond well¹⁴ and were used for this experiment. DNA was prepared from cells mainly in early S, middle S, and G2 phase (Fig. 4), and fractionated by CsCl gradient centrifugation. The high-density material was recovered and investigated by electron microscopy. The DNA preparations from S phase cells contained about three times larger amounts of single-stranded material than the preparation from cells mainly in G2 phase (Table 1). These data

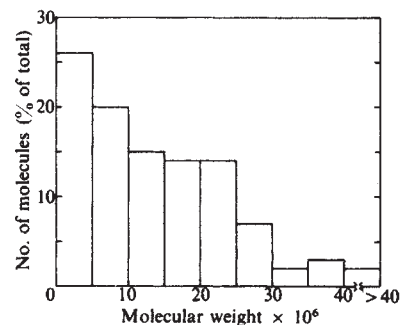


Fig. 3 Size distribution of long single-stranded DNA regions. Molt-4 DNA was fractionated by neutral CsCl and neutral glycerol density gradient centrifugations, and the material sedimenting at $>50\text{S}$ was investigated. Both entire single-stranded molecules and single-stranded regions in partly double-stranded molecules were included, and 100 molecules were measured.

agree with previous findings^{3,4,8} that single-stranded DNA is primarily (or exclusively) found in S phase cells.

The subcellular distribution of the single-stranded DNA was investigated by preparation of Raji cell nuclei by Nonidet 40 treatment as described previously¹⁵. High molecular weight DNA preparations were made in parallel from both whole cells and isolated cell nuclei, and after CsCl density gradient centrifugation, the DNA density fractions $1.720\text{--}1.740\text{ g cm}^{-3}$ was examined by electron microscopy. In both these preparations 10–15% of the high molecular weight material was single-stranded DNA. Thus, we conclude that most or all of the long regions of single-stranded DNA described here is of nuclear origin.

When Molt-4 cells were pulse-labelled with $10\text{ }\mu\text{Ci } ^3\text{H-thymidine}$ per ml 30 min before collecting the cells for preparation of DNA, the total DNA had a specific radioactivity of 4,000 c.p.m. per μg . After enrichment of large single-stranded DNA molecules by CsCl and glycerol gradient centrifugation as above, a fast-sedimenting fraction representing about 0.3% of the total DNA and containing 50% single-stranded material was obtained. This DNA had a specific activity of about 3,000 c.p.m. per μg . Although the estimate of the DNA concentration is only semi-quantitative, these data strongly indicate that the large

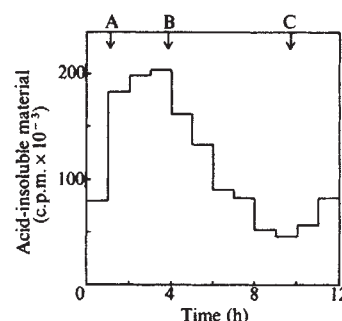


Fig. 4 DNA synthesis in synchronised lymphoid cells. Cells (60×10^5) of the Raji line were propagated in stationary suspension culture and synchronised by the double thymidine blocking technique as described by Hampar *et al.*¹⁴. After release from the block, DNA synthesis was monitored by 1-h pulse-labelling of aliquots of 5×10^5 cells with $5\text{ }\mu\text{Ci } ^3\text{H-thymidine}$. At the three time points indicated by arrows, 15×10^6 cells were collected and used for the preparation of high molecular weight DNA. Each DNA solution was fractionated by neutral CsCl density gradient centrifugation⁹. Fractions of density $1.720\text{--}1.740\text{ g cm}^{-3}$, which represented about 2% of the DNA in the gradient, were pooled, dialysed and investigated by electron microscopy (see Table 1).

single-stranded DNA regions do not represent newly synthesised DNA, again confirming previous results^{3,5}.

Methods used previously¹⁻⁵ to isolate single-stranded DNA from mammalian cells yield larger amounts of material suitable for further characterisation. However, the approach used here reveals the unexpected length of much of this single-stranded DNA. It seems unlikely that such long regions could be due to DNA destabilised by transcription or DNA repair, or to DNA sequences serving as regulatory signals. Note that during replication of adenovirus DNA in cell nuclei, the replication process occurs in a completely asymmetric manner with the generation of single strands of entire parental virus DNA as replication intermediates^{16,17}. Single-stranded DNA of the size range described here can thus exist in cell nuclei without being degraded. Also, mitochondrial DNA is synthesised in an asymmetric manner with the temporary generation of single-stranded regions of parental DNA¹⁸.

Recently, it has been found that both adenovirus DNA and mitochondrial DNA are replicated by DNA polymerase γ (refs 19, 20), whereas most of the nuclear DNA is replicated by DNA polymerase α in a symmetrical, bi-directional way^{21,22}. Both these polymerases are present in the nuclei of uninfected cells and are thought to act in DNA replication, as they are present in increased amounts in S phase cells²³. It seems that two fundamentally different modes of DNA replication may occur in human cells, symmetric synthesis catalysed by DNA polymerase α and asymmetric synthesis catalysed by DNA polymerase γ (ref. 19). The long single-stranded DNA regions visualised here may then represent displaced strands of parental DNA in asymmetric DNA synthesis.

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Codon-anticodon interaction at the ribosomal peptidyl-site

VARIOUS models have been proposed to explain the interaction between an aminoacyl-tRNA molecule and the ribosomal A and P sites during one elongation cycle of protein biosynthesis¹⁻⁷. In general all these models agree as to the existence of at least two tRNA binding sites on the 50S subunit, the A and P sites. These models, however, do not agree on the important question of whether mRNA-tRNA (codon-anticodon) recognition occurs exclusively in the A site or in both A and P sites. Previous experimental evidence adduced in favour of either hypothesis has usually been based on analysis of the effects of various antibiotics on the partial reactions of protein synthesis. However, molecular mechanisms of action of such antibiotics are still poorly understood, and definite conclusions as to which type of model is correct cannot be based solely on such data. Here we show that factor-mediated binding of aminoacyl-tRNA (aa-tRNA) to the ribosomal A site is dependent on the presence of both an mRNA codon in the P site and of a tRNA which recognises that codon. Thus binding of a ternary complex EF-Tu · aa-tRNA · GTP to the ribosomal A site occurs only if the P site is occupied by a tRNA for which codon-anticodon interaction is maintained (for a preliminary report see ref. 8).

Initially we studied the binding of Phe-tRNA to ribosomes in the presence of 8 mM Mg^{2+} using oligouridylylate templates with a chain length of between 3 and 10: the chain length dependence was found to be different in the presence and absence of EF-Tu (Fig. 1). Considerable nonenzymatic Phe-tRNA binding to 70S ribosomes was observed (even at this low Mg^{2+} concentration) with each of the eight oligouridylylates investigated. Even with the triplet UUU, 3 pmol of Phe-tRNA were bound to 100 pmol 70S ribosomes, and only a small increase was observed with the longer oligouridylylates (Fig. 1). In striking contrast to nonenzymatic binding, no EF-Tu mediated binding of Phe-tRNA was observed unless the chain length of the oligouridylylate was equal to or longer than 6 (Fig. 1).

The requirement for $(U)_6$ for enzymatic binding of Phe-tRNA suggests that two tRNA molecules form a codon-dependent complex with the ribosome. Aminoacylated Phe-tRNA^{Phe}, exclusively present as preformed ternary complex, bound into

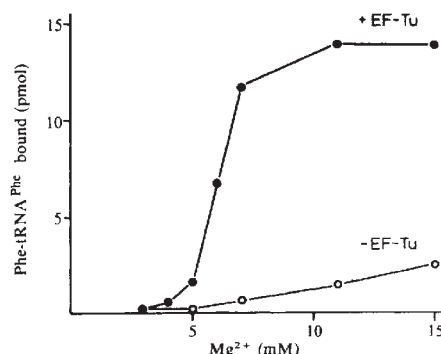


Fig. 1 Enzymatic (●) and nonenzymatic (○) binding of Phe-tRNA to 70S ribosomes in the presence of oligonucleotide $(U)_3$ to $(U)_{10}$. The binding of Phe-tRNA to 70S ribosomal tight couples⁹ was assayed as follows: 75 μ l of a mixture containing 20 mM Tris-HCl (pH 7.5), 50 mM NH_4Cl , 10 mM 2-mercaptoethanol 8 mM magnesium acetate, 100 pmol 70S ribosomes and 25 nmol oligouridylylate¹⁰ was preincubated for 5 min at 37 °C and cooled on ice. For nonenzymatic Phe-tRNA binding the sample was then supplemented with 25 μ l of a mixture containing the same salt concentrations as above, together with 100 pmol bulk [³H]Phe-tRNA (2 Ci mmol⁻¹, charging degree 2%). When enzymatic Phe-tRNA binding was to be measured, 300 pmol EF-Tu¹¹ and 0.2 μ mol GTP were included. The mixtures were incubated for 10 min at 37 °C. Ribosomal binding of Phe-tRNA was measured by the Millipore filter technique¹².

Table 1 Binding of Phe-tRNA to 30S ribosomal subunits with oligonucleotide (U)₃ to (U)₇ in the presence and absence of EF-Tu

Oligonucleotide	Phe-tRNA ^{Phe} bound (pmol)	
	EF-Tu absent	EF-Tu present
(U) ₃	4.3	4.0
(U) ₄	3.9	3.9
(U) ₅	4.1	4.2
(U) ₆	4.4	4.5
(U) ₇	4.8	5.0

Experimental conditions as in Fig. 1 except that the binding was carried out with 100 pmol 30S ribosomal subunits at 16 mM Mg²⁺ with an incubation for 30 min at 0°C.

the A site of 70S ribosomes, whereas the P site was exclusively occupied by deacylated tRNA^{Phe}. The evidence for this suggestion came from experiments which showed that no diphenylalanines were formed (as judged by peptide analysis) and none of the Phe-tRNA bound to the ribosome reacted with puromycin, that is, aminoacylated Phe-tRNA did not bind into the P site (data not shown).

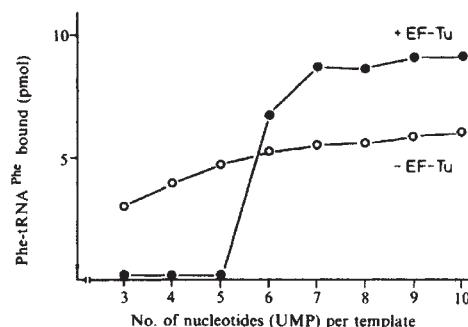
In contrast, the degree of Phe-tRNA binding to 30S ribosomal subunits is independent of the oligouridylylate chain length within the range of 3 to 7 (Table 1) and occurs to the same extent, regardless whether EF-Tu is present or not (Table 1). Thus each of the specific effects of EF-Tu described above requires 70S ribosomes.

The experiments with hexauridylylates (Fig. 1) provided evidence that occupancy of the ribosomal P site by an uncharged tRNA was a prerequisite for subsequent enzymatic binding of Phe-tRNA^{Phe} to the 70S A site. To demonstrate that the occupation of the P site had to be codon-specific to permit subsequent enzymatic tRNA binding to the A site, the hexanucleotides AUGUUU, GUAUUU and UAUUUU were synthesised and used as templates. Four pure deacylated tRNAs (tRNA^{Met}, tRNA^{Val}, tRNA^{Tyr} and tRNA^{Lys}) were tested for this stimulatory activity. The results, summarised in Table 2, confirmed our predictions. EF-Tu-mediated Phe-tRNA^{Phe} binding was dependent on the presence of the tRNA cognate to the 5'-codon (Table 2). The combination of tRNA^{Met} and AUGUUU gave the highest degree of Phe-tRNA^{Phe} binding, which was in each experiment above 40%. This effect was absolutely dependent on the presence of the 5'-AUG codon, since the stimulatory effect of tRNA^{Met} was not observed with either GUAUUU or UAUUUU as templates (Table 2). This result is of special importance, since tRNA^{Met} has a high intrinsic affinity for the P site¹³. It excludes the possibility that the observed enhancement of enzymatic Phe-tRNA^{Phe} binding to the A site with AUGUUU was only a consequence of the occupation of the P site by the initiator tRNA without concomitant codon-anticodon interaction. This was confirmed when use of tRNA^{Val} and tRNA^{Tyr} gave qualitatively similar codon-specific effects (Table 2). The effects of these two tRNAs on

Table 2 Enzymatic binding of Phe-tRNA^{Phe} to 70S ribosomes with AUGUUU, GUAUUU and UAUUUU is strictly dependent on the tRNA which is cognate to the first codon

Deacylated tRNA	Phe-tRNA ^{Phe} bound (pmol)		
	AUGUUU	GUAUUU	UAUUUU
None	0.7	0.9	0.7
tRNA ^{Met}	10.0	0.6	0.9
tRNA ^{Val}	0.5	4.5	0.6
tRNA ^{Tyr}	0.6	0.8	3.0
tRNA ^{Lys}	0.5	0.6	0.5

Reaction conditions were as described in Fig. 1 except that the incubation mixtures contained 25 pmol 70S ribosomes, 25 pmol of pure [³H]Phe-tRNA^{Phe}, 100 pmol EF-Tu, 5 nmol of hexanucleotide¹⁰ and 50 pmol of pure deacylated tRNA as indicated.

**Fig. 2** Mg²⁺ dependence of the enzymatic (●) and nonenzymatic binding (○) of the Phe-tRNA^{Phe} to 70S ribosomes mediated by AUGUUU. Experimental conditions as in Table 2 except that the reaction mixtures contained 2 nmol AUGUUU and 50 pmol of pure deacylated tRNA^{Met}.

enzymatic Phe-tRNA^{Phe} binding were less marked than those seen with tRNA^{Met}, probably because of the lower affinity of deacylated non-initiator tRNA species for the ribosomal P site. tRNA^{Lys} had no effect (Table 2), which is as expected since the cognate codon for this tRNA (AAA) was not present in the templates.

Codon-specific occupation of the ribosomal P site with deacylated tRNA allows the subsequent binding of an aa-tRNA into the ribosomal A site to be studied in isolation, since peptidyl-transfer cannot occur in any of the systems described above. For AUGUUU/tRNA^{Met} the active role of elongation factor EF-Tu in promoting aa-tRNA binding into the A site is not restricted to a unique Mg²⁺ concentration (Fig. 2). Negligible binding of Phe-tRNA^{Phe} to the ribosomes was observed from 5 to 15 mM Mg²⁺ in the absence of EF-Tu. However, in the presence of EF-Tu (and hence the ternary complex) maximal binding of Phe-tRNA^{Phe} was reached at and above 7 mM Mg²⁺ (Fig. 2).

Our findings are in accordance with the observations of others, who found a similar stimulation of enzymatic Phe-tRNA binding when the P site of poly (U)-programmed ribosomes was occupied either with *N*-acetyl-Phe-tRNA¹⁴ or with peptidyl-tRNAs^{15,16} but our data conflict with the conclusion¹⁷ that the states of occupation of A and P sites are independent of one another. The latter experiments were, however, performed under nonphysiological conditions, namely at 20 mM Mg²⁺ and without elongation factor EF-Tu.

The experiments reported here prove that EF-Tu mediated Phe-tRNA^{Phe} binding to 70S ribosomes is possible if (1) the template (N)₁-UUU has a minimum chain length of 6 (Fig. 1) and (2) a tRNA is present which fits the 5'-neighbouring NNN codon. Non-cognate tRNAs are ineffective, even if they bind tightly to 70S ribosomes in the absence of their codon (for example, tRNA^{Met}; Table 2).

These observations can be explained by assuming that in physiological conditions (in the presence of EF-Tu and at low Mg²⁺ concentrations) the A site of the 70S ribosome can accommodate aa-tRNA only within the ternary complex EF-Tu · aa-tRNA · GTP and, even then, only when the P site is occupied by mRNA-cognate tRNA. Furthermore, the experiments strongly suggest that the A site only exists as such when the P site is so occupied. Further experiments will be required to show whether the codon-selected tRNA in the P site acts only as an effector molecule, which 'opens' the A site, or whether tRNA-tRNA interactions occurring within the ribosome are also involved.

Regardless of this uncertainty, our results show that codon-anticodon interaction is maintained by the P site-bound tRNA. Our results are consistent with conclusions drawn from recent experiments of Wurmbach and Nierhaus¹⁸. These authors analysed a series of purified deacylated tRNAs for their ability to block the P site binding of *N*-acetyl-Phe-tRNA to poly

(U)-programmed 70S ribosomes and to direct the same tRNA to the A site, and were able to show that only the cognate tRNA was effective.

The AUGUUU/tRNA^{Met} system may serve as an ideal and simple model system for investigations concerned solely with the binding of a ternary complex to the ribosomal A site, since the system is not dependent on the presence of initiation factors. Furthermore, the complexes of 70S ribosomes carrying Phe-tRNA^{Phe} in the A site can be purified on a large scale by sucrose gradient centrifugation without loss of pre-bound Phe-tRNA (unpublished results). Thus the system may be useful for the study of the effects of some antibiotics and also for physicochemical investigations, such as fluorimetric studies, with tRNAs or templates specifically modified with fluorescent probes.

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Atmospheric lead pollution of grass grown in a background area in Denmark

LEAD in the atmosphere of the Northern Hemisphere originates mostly from lead-alkyl petrol additives emitted from car exhausts¹. A small proportion of this lead—estimated at about 10%^{2,3}—is deposited within 50–100 m of the road while the rest is presumably transported long distances before deposition. There are reports of increasing concentrations of lead in roadside vegetation with decreasing distance from the edge of the road^{4,5}. This apparent lead contamination ceases beyond 100–200 m from the road, and it is said therefore that airborne lead is not a significant source of lead in crops in general^{5–7}, although there is circumstantial evidence to the contrary^{8,9}. We report here, however, that the lead taken up by grass in a remote rural area is predominantly from the atmosphere.

Table 1 summarises evidence of airborne lead in background areas far from roads and towns throughout the world^{10,11}. If airborne lead were present everywhere, aerial deposition on vegetation could account for part of the lead content found in plants. Lead concentrations reported for protected plant organs are normally very much lower than for unprotected parts. The average rate of deposition of lead from the atmosphere in Denmark is about 1 mg m⁻² per 30 d¹², which is much greater than the rate of accumulation of lead in an average crop, 0.1–0.2 mg m⁻² per 30 d. To determine the uptake of lead by

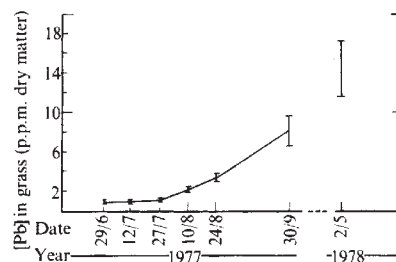


Fig. 1 Lead concentration in grass during growing season, p.p.m. dry matter. Vertical bars indicate $\pm\sigma$ on observations.

plant roots, specific activity (counts per 10³ s per μ g Pb) was measured in soil labelled with ²¹⁰Pb and compared with the specific activity in the plants. Any difference in specific activity in the plants had to be the result of uptake of nonradioactive lead by the plants. The site of our experiment was the experimental station at Højbakkegaard in Taastrup, west of Copenhagen. We know of no industrial sources of lead contamination within a radius of 5 km. The E66 highway runs east–west 1 km to the south (22,000 cars per 24 h), and the area is surrounded by agricultural farmland and forests.

We carried out a pot experiment involving three different soils and a crop of Italian rye-grass (*Lolium multiflorum* Lam.). Pots were fertilised with normal amounts of N, P and K at the beginning of the season to give a normal growth rate. Six cuttings were taken during 1977 and one in 1978. Soils were labelled with ²¹⁰Pb (0.02 mCi per kg soil) 18 months before sowing. During this period the soils were mixed thoroughly several times and wetted to obtain isotopic equilibrium.

The surface area of the polyethylene pots was 250 cm², and the soil was 20 cm deep. Below the soil was a layer of 5 cm of acid-washed quartz on top of filter paper. Drainage was provided through a hole (1 cm diameter) at the bottom of the pot. The pots were sunk into a lawn, so that their edges were level with the soil surface, and placed on a layer of sand to secure drainage. After the experiment no roots could be seen emerging from the drainage hole in the pots. The height of the lawn was kept the same as that of the grass in the pots.

During the growing season the bulk precipitation of lead and the content of lead in the ambient air around the experimental station were measured. The rain was collected in a polyethylene funnel/bottle system and acidified with HNO₃ as preservative. Aerosols were sampled on an OECD standard sampler¹⁵ modified with a 5-cm filter (Whatman no. 1), and sampling about 2 m³ d⁻¹. The filters were extracted in 1M HNO₃ (boiling) before analysis.

Plant samples were dried in paper bags at 50 °C, and digested in HNO₃: HClO₄ (10:1). Soil samples were treated with three extractants to assess differences in isotopic labelling (1:1 HNO₃, 0.02 M EDTA or ammonium acetate (pH ~4, 8)). The determinations of lead in prepared sample solutions were made on atomic absorption spectroscopy with graphite atomiser¹³. Solvent extraction (DDDC in xylene) was used to eliminate interference¹⁶. The activity of ²¹⁰Pb in digests or extracts was measured by crystal scintillation counting.

A major objection to using radioactive tracers in a soil

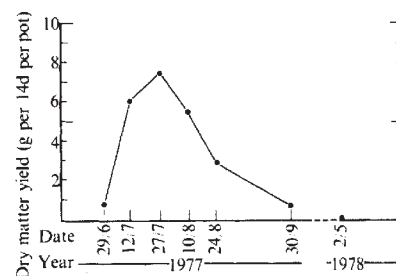


Fig. 2 Rate of dry matter yield, expressed as grams of dry matter (collected) per 14 days per pot.

Table 1 Lead concentrations in air and lead deposition, and mean values for local background levels

Country and Locality	Period	Lead in air (ng m ⁻³)	Lead deposition (mg m ⁻² per 30 d)	Ref.
USA				
Intermediate non urban	1966-67	96		10
Proximate non urban	1966-67	210		10
Belgium				
Botrange	1972-75	212		11
Holland				
Background	1974-76		0.5	12
Rural area	1974-76		1.0	12
UK				
Plymilton	1976	46	1.4	13
Denmark				
Jutland, Tange	1973	65		*
Rural areas, 12 stations	1976		0.8	14
Pot experimental site	1977	132	0.9	This work
Taastrup				

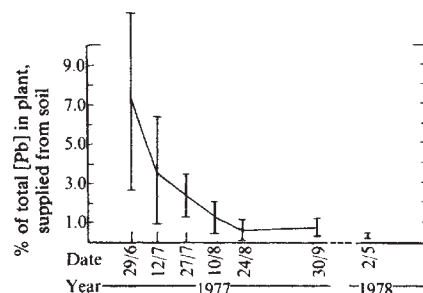
* The Danish Meteorological Institute, Air Pollution Section (1973).

experiment is the uncertainty of isotopic equilibration. However, as three methods of soil extraction gave the same specific activity, we believe that the added ²¹⁰Pb had attained true isotopic equilibrium with the indigenous lead. Further, if the added ²¹⁰Pb was less strongly bound than the indigenous lead, the observed specific activity in the grass would be too high, leading to an underestimation of the atmospheric lead supply to the plant.

The characteristics of the soils are given in Table 2. Average lead deposition and the average ambient air concentration are shown in Table 1. The values observed at the experimental site are higher than those found at remote sites, but they are within the range found in rural areas of western Europe and the US.

The total lead concentrations in the plant material that had been exposed to the atmosphere varied from ~0.6 to 8 p.p.m. dry matter for plants grown in the summer, while concentrations were considerably greater for those grown during the winter. The range of lead concentrations found is quite similar to the values summarised by Höll and Hampf¹ for grasses sampled in background locations. In research on the application of sludge to the land conducted in cooperation with three state research farms in Denmark we have found that the concentration of lead in grass from untreated fields is in the range 1-6 p.p.m. of dry matter, most values averaging around 2 p.p.m.

The observed increase in the lead concentration during the growing season is consistent with the findings of other authors^{6,14}. This phenomenon may be explained as a decreasing dilution of the atmospherically supplied lead with newly grown

**Fig. 3** Root uptake of lead expressed in percentages of total lead supply to plant. Vertical bars indicate $\pm\sigma$ on observations.

grass, due to the decrease in growth rate after the midsummer peak.

Figure 2 shows the average yield of dry matter per pot per 14 days. Yields are comparable to normal yields on grassland, being of the order of 6-10 ton of dry matter per hectare per season. The specific activity of lead in the soil was 1,500-3,100 counts per 10³ s per μ g Pb, and in the grass 4-200 counts per 10³ s per μ g Pb.

The total concentration of lead in the grass, and the calculated percentage of lead in the grass coming from the soil did not differ significantly between the three soils used in spite of differences in pH and texture. Figures 1-3 are thus the averages of results obtained for the three soils in six pots.

The percentage of lead in the grass crop originating from the soil was calculated as:

$$\% \text{ Pb}_{\text{soil}} = \frac{\text{Specific activity of Pb in crop}}{\text{Specific activity of Pb in soil}} \times 100$$

Figure 3 shows that it was 0.2-10% of the total lead content of the green parts of the grass. This result will not be greatly affected by the earlier discussed possibility of isotopic inequilibrium in the soils, as an underestimation of the atmospheric contribution is hardly important when it is more than 90%. Thus deposition of atmospheric lead on the plant surface was responsible for between 90 and 99% of total lead content in the grass in this typical background area in Denmark.

We thank the staff of the experimental station at Taastrup for valuable help. This project was financed in part by the Danish Agricultural and Veterinary Research Council.

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Table 2 Characteristics of soils in the pot experiment

Origin and type of soil*	Rønhave (loam)	Askov (sandy loam)	Lundgaard (sandy)
Clay % <0.002 mm	16	11	3
Silt % 0.002-0.02 mm	21	12	4
Fine sand % 0.02-0.2 mm	37	38	26
Coarse sand % 0.2-2 mm	24	37	65
Organic matter %	2	3	2
CEC mEqv per 100 g dry matter	13	12	5
pH _{CaCl2}	6.6	6.3	4.6
Total lead p.p.m. in dry weight	17.3	14.4	11.1

* The soils are arable top soils (0-25 cm).

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reviews

Defining sociobiology

Richard Dawkins

Sociobiology: Sense or Nonsense? By Michael Ruse. Pp. 231. (D. Reidel: Dordrecht, The Netherlands, and Boston, Massachusetts, 1979.)

WHAT on earth is sociobiology? If philosophers have a role to play in science it presumably includes clearing up semantic confusion. Michael Ruse is a distinguished philosopher of biology who would agree: "I am a philosopher, and for me, in John Locke's great words, 'it is ambition enough to be employed as an under-labourer in clearing the ground a little and removing some of the rubbish that lies in the way of knowledge . . .'" (p164). Yet, after reading this sensible and pleasantly written book, I remain unable to answer the question with which I began. What is sociobiology and who are the sociobiologists?

Well, if anybody is a sociobiologist it is E. O. Wilson, so his definition must be taken seriously: ". . . the systematic study of the biological basis of all social behavior" (*Sociobiology: The New Synthesis*; Harvard University Press, 1975). A neutral discipline then, like history or geology. Geology or history as such cannot be true or false, yet Ruse debates the "truth" of sociobiology, and whether it is sense or nonsense. I sympathise. However neutral Wilson's definition may be, the widespread identification of "sociobiology" with a school of thought is an undeniable fact of current biological sociology, a linguistic oddity whose only recent biological parallel is the popular transmutation of "ecology" from a scientific discipline to a political opinion.

What, then, is this cluster of ideas, this bundle of concepts that have become linked in people's minds both with each other and with the name sociobiology? The answer is complicated because it depends on whether you read biologists or social scientists. Ruse has read both and absorbed both meanings, apparently without realising it. I will return to the social scientists' meaning later. The biological cluster is well represented by

section headings in Ruse's chapter on "The Sociobiology of Animals": kin-selection, parental investment, reciprocal altruism, evolutionarily stable strategy. How the cluster came to be linked in our collective mind is not clear. Wilson's *Sociobiology* can hardly be the common denominator, for, although these concepts are mostly mentioned there, so is almost everything else. As befits his own definition, Wilson rightly devotes more space to the comparative study of social systems and their correlation with environmental variables, important topics which seem to be absent from the mysterious cluster and absent from Ruse's book.

My own slightly irreverent epitome ("sociobiology is the branch of ethology inspired by W. D. Hamilton") leaves the timing unexplained. A little research in *Science Citation Index* shows that Hamilton's now famous papers in the 1964 *Journal of Theoretical Biology* were seldom cited in the nine years up to 1973. Then, in 1974, the year before *Sociobiology*, the curve suddenly turns up sharply and has soared (almost exactly exponentially) ever since. When concepts that have been ignored a long time suddenly group themselves together and proliferate like fire through the imaginations of scientists, then philosophers and historians of science should have interesting things to say. Unless, that is, they are lured away from constructive analysis by a preoccupation with destructive criticism and the supposed need to reply to it. In this respect Ruse's book is a disappointment.

It is, however, not all disappointing. Ruse may not define his subject, but he does a good job of discussing it, whatever it is. He writes in an easy, unpretentious style, and his aim is clearly to communicate with his readers rather than to show off to them. "Of course, if I get my biology wrong I expect to be criticised by biologists; but that is nothing to what I expect from philosophers if I get my philosophy wrong . . ." This particular biologist has no complaints about the chapter on "The Sociobiology of Animals"; it might have been written

by a biologist. Oddly enough, my most serious worry is over an issue that could be called philosophical, the one known by the cliché of nature and nurture.

The trouble begins to surface in the chapter on "The Positive Evidence" (for human sociobiology), where he makes the following strange remark: "I take it that the problem at issue is the extent to which human behaviour, specifically social behaviour, is essentially a function of the genes, where, by this, is meant that the behaviour will manifest itself under normally occurring environments without the need for any special learning input". We have now moved on to the social scientists' understanding of "sociobiology", which has nothing to do with Wilson's neutral definition (unless perhaps you think "biological" means "genetic"), nor with my "mysterious cluster". Sociobiologists are apparently supposed to believe that human behaviour is governed by genes as opposed to learning (I even recently heard Sir Cyril Burt quoted, not by Ruse of course, as an example of a sociobiologist!).

Let me briefly put my finger on this muddle, for it is a common one and I know it well. Whoever the sociobiologists may be, nobody would deny that they are interested in behaviour as Darwinian adaptation. In order to talk about the Darwinian evolution of anything we have to postulate the sometime existence of genetic *differences* between individuals — otherwise there would have been nothing for selection to choose between. This does not mean there has to be genetic variance any longer: any characteristic that has been strongly favoured by selection is likely now to have low heritability, the genetic variance having been used up. More importantly, a genetic contribution to differences is not the same as (indeed, it has nothing whatever to do with) behaviour manifesting itself "without the need for any special learning input". As Konrad Lorenz and others have emphasised for years, learned behaviour must itself be subject to Darwinian, therefore genetic, selection. Happily, Ruse does not fall for the even more extreme muddle, beloved of

extreme hereditarians and their unwitting allies among "scientific radicals", of equating genetic variance with irrevocable, unswerving genetic determinism.

Ruse is rather good at dealing with such people. In America there are some genuinely good scientists who have severely criticised sociobiology, and we have to listen to them. Ruse does so, understands them, agrees with them in places, but finally remains largely unimpressed. He also gives some reassuring pats to the ruffled feathers of social scientists who fear imminent takeover by a rapacious biological imperialism.

I have concentrated on places where the book could have been improved, but there is good in it and I hope it will

be read by those who have managed to retain an interest in this rather over-publicised controversy. Ruse's sense of balance and agreeable style are well shown in his closing sentences: "... I am far from convinced that human sociobiologists have yet made their case. What I do plead is that their sins are not as grave as their critics argue. Human sociobiology should be given the chance to prove its worth. If it cannot deliver on its promises, it will collapse soon enough; but if it does prove viable, then its success could pay scientific dividends of the highest order". Who will disagree? □

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Protein synthesis inhibitors

Inhibitors of Protein Biosynthesis. By David Vazquez. Pp. 312. (Springer: Berlin, Heidelberg and New York, 1979.) \$32.50.

No matter how hard you try to keep up with the literature in this field, you usually find it slipping away from you. Well, here's a good chance to catch up with all of it. In this comprehensive survey David Vazquez cites 1017 references (I think), of which a mere 100 originated from his laboratory, and pulls so many rabbits out of the hat that one feels he could have directed *Watership Down*.

The book is bulging with information including the structures, empirical formulae, molecular weights (many thanks) and synonyms of antibiotics together with the names of the producing organisms. The subject of synonyms is particularly intractable and this reviewer is now glad to know that multithiomyacin and nosiheptide are the same thing, to say nothing of anhelmycin and hikizimycin. All your old favourites are here and, when you turn to the appropriate Figure, you'll probably find they have a few cousins you didn't know about. It's inevitable (isn't it?) that something should have been omitted, but I'll have to check carefully before I complain that pulvomycin should have been included with the rest of the kirromycin group in Fig. 42. (You never know, it could be an alias for efrotomycin!)

All the alkaloids are in here too and when botanists have finished talking about them as being herbivore-repellents, or nitrogen excretion products, or whatever, they should note just how many of them are ribosome-inhibitors. The same is true for various other plant products known more generally as lectins and agglutinins.

Of course the main aim of this book is to deal with the action of inhibitors of protein synthesis. So, if you want to know what others think they know about the mode of action of any antibiotic or drug, David Vazquez will tell you. For this reason the book does not read like an essay; with so many facts and figures (61 of the latter) it is meant to be used as a reference book. As such it should find a place in research laboratories and in reference collections, although it cannot be recommended for students. However, no-one working in the fields of antibiotic action, protein synthesis or ribosome structure-function analysis should be without it. I warmly recommend it.

Eric Cundliffe

Interpreting visual motion

The Interpretation of Visual Motion. By Shimon Ullman. Pp. 229. (MIT Press: Cambridge, Massachusetts, and London, UK, 1979.) \$17.50; £11.40.

THIS book is a slight revision and extension of Ullman's doctoral thesis researched at the Artificial Intelligence Laboratory of MIT. Various parts of it have also been published independently (in somewhat different form) in various journals (*Perception*, *Perception and Psychophysics*, *Proc. R. Soc. London*). Nevertheless, the book is worth possessing in its own right if you are an experimental psychologist or an artificial intelligence researcher interested in vision, especially in motion perception, primarily because of its special methodological qualities.

Ullman investigates the phenomenon of apparent (or phi) motion, the illusion enabling us to watch television or movie pictures despite their discrete presentation. The phenomenon is conceptualised as a computational problem and is divided into two parts, each of which is studied independently. Solving a computational problem includes specifying the goal of the computation, and then investigating possible (and plausible) methods for attaining the goal. A theory of what is being computed is thus an integral part of the understanding of the information processing system being studied, in this case the human visual system. The phi phenomenon is seen to establish the correspondences between the elements in two successive "snap-shots" of a scene, and then interpreting the resulting displacements as motions of the objects.

Ullman shows that the correspondence computation amounts to computing the so-called minimal mapping, the match that minimises costs over the covers of the graph of possible (probability weighted) pairings. He shows that the initial candidates for a set of possible pairings for a given element in one snapshot can be

restricted to elements (in the second snapshot) lying within some limited distance radius of this element. This means that the correspondence process can be effected by a simple network of locally competing processors of uniform structure. Furthermore, Ullman shows that the domain of the computation is a "token space" (D. Marr's "primal sketch": *Phil. Trans. R. Soc. B* **275**, 483-524: 1976), a symbolic representation consisting of blobs, line segments, edge fragments and the like, which is the result of pre-processing the raw image intensity distributions.

The next stage consists of interpreting the displacements obtained. Ullman develops the notion of a "rigidity test", operating locally on a nucleus of elements and leading to decisions about whether the displacements associated with the nucleus can possibly represent (and result from) a rigid object in motion. The analysis is based on his "structure-from-motion" theorem which states that the structure (relative position) of four non-planar points is uniquely recoverable from three orthographic projections. Such an interpretation does not assume any recognition of, or familiarity with the objects in question.

Finally, Ullman discusses "motion-from-structure" situations in which motion is established as a "filling-in" process linking two objects recognised as being an instance of the same object in the two successive static snapshots. He compares the two motion-analysing mechanisms and argues that various interpreting processes (analysis of static "snapshots", "motion-from-structure", "structure-from-motion", and so on) all contribute to one common representation and that they must compete or co-operate to produce the final result, the percept.

This monograph is clearly written. Within the limits he has set for himself, Ullman has achieved a great deal. This is an excellent and important piece of work.

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Human information processing

Strategies of Information Processing. Edited by G. Underwood. Pp.455. (Academic: London and New York, 1978.) £20.

In the 1950s and early 1960s scientists who discussed selective attention, perceptual discrimination, reading and the choice of responses called themselves Human Experimental Psychologists. They were earnest people prone to strange methodological obsessions. They used models derived from the theory of simple, passive communications systems and metrics derived from information theory and communications theory. Nowadays the same people are relaxed and expansive. Most have abandoned interest in methodology or metrics and many even in experiments. Nowadays we are Cognitive Psychologists. Among very young research workers it is a dogma that this euphoric transformation (including, incidentally, the titling of three prestigious journals, at least two large departments of psychology, four honorific chairs and an Open University course) came about as a result of a single book, Ulrich Neisser's *Cognitive Psychology* published in 1967. Older workers are embarrassed to enjoy their new liberties without knowing what they must believe to earn them. They find it strange that a very slight book should be credited with a Cognitive Revolution and, by and large, those of us who still do research do the same things we have always done under new titles.

The middle-aged are not prone to rocking boats - even Ships of Fools - so it is right that complaints should come from a young research worker, Geoffrey Underwood, who has edited a collection of 12 essays under the title *Strategies of Information Processing*. Underwood takes his role as challenger of the new establishment as seriously as if he were writing in 1958. He insists that his book is a "statement of faith" (Preface, p1). Apparently he believes that classical models of human information processing may be revived within the context of models drawn from the theory of self-optimising control systems. He feels that these rehabilitated models will provide means for empirical investigations of intention, and purpose, and even perhaps sensible approaches to problems of consciousness. That is they will simultaneously teach Cognitive Psychologists to do proper experiments and disarm their critics (such as Gauld, Shotter and the anti-empiricists) who believe that the most important problems of human psychology are inaccessible to laboratory investigation.

Older research workers may be expected to brighten at this news. The dear

familiar models of our youth are to be justified as the basis of the next conceptual advance. We have always lived beyond the current fashion without knowing it.

The publication of these papers will not quite achieve this. Critics who objected to "mechanistic" psychology in the guise of simple communications models are not likely to be placated by descriptions of purpose in terms of loops and branches of computer programs or conceptions of intentionality paraphrased as the search for the optimum among possible paths in a decision tree.

Control theory models have been common in ethnology since the late 1960s and excellent summaries of their applications by MacFarland, Oatley and Toates have formed the basis of undergraduate courses for some years. The omission of recent work on ethological systems is a sad omission from this book. There is little about the contributions which suggests any striking novelty of approach - or even any daring retrogression. Neville Moray now provides a clear and useful summary of the lectures on optimisation of informational selectivity which he has given over the past six years. Max Coltheart usefully reviews his recent thinking about lexical access during reading. A review of hemispheric asymmetry effects by M.P. Bryden provides an updated bibliography of the unreliable literature in a field recently very popular among Cognitive Psychologists. The inclusion of the word *strategy* in the titles of contributions seems to reflect loyalty to the editor rather than any worked-out change in theoretical ap-

proach. For example, C.I. Howarth reviews his own, excellent and sadly neglected, work on motor skills under the title "Strategies in the Control of Movement". There are useful digests of research on speech perception by Cole and Jakimek and on eye-movements during reading by Alan Kennedy. One paper certainly has a novel theme. John Fox provides an exegesis of hypothetical neurology of vision proposed by Dr. Marr. This work is fascinating, and original, but Marr's own papers are more lucid, much richer in their implications, more rigorous in argument and now generally available. Fox's paper is cheerful and entertaining, but why is it necessary for him to act as a sort of intellectual Basil Brush on behalf of a very talented colleague?

In sum, this is a very handy reference work, invaluable for teaching courses to third-year undergraduates. It does repeatedly stress the point that human beings must be considered as active rather than passive self-optimising systems and it illustrates this emphasis with data from a wide range of experimental situations. Librarians and teachers will find this a useful book to buy, but active research workers will be busy with the current issues of journals and have read it all long ago.

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Biological transport

Mechanics and Energetics of Biological Transport. By E. Heinz. Pp. 159. (Springer: Berlin, Heidelberg and New York, 1978.) DM 49; \$24.50.

THE swift development of biological transport as a discipline in its own right over the past two or three decades has proved something of a phenomenon, and has brought in its wake the publication of a near-avalanche of books. Predictably, the majority of these are edited proceedings of meetings or collections of review articles, and suffer from the lack of coherence both in style and content peculiar to this particular genre. Some notable exceptions are the well-known treatises of Stein, Schoffeniels, Kotyk and Janáček, and more recently Christensen, each of which is written from a highly individual standpoint and intended as an all-embracing treatment. What is missing at present is a comprehensive analysis devoted entirely to the energy transactions of the transport process, which would draw together material scattered in a forest of original reports and reviews.

In this regard, the monograph under consideration (offset printed from a typed manuscript) presents, first and foremost, a concise compilation of *kinetic* models and formulae. Still, a commendably broad range of topics is included, covering one-flow and two-flow systems, along with an approach to the description of coupled transport in terms of nonequilibrium thermodynamics. Among the topics touched on in summary fashion are diffusion, flux ratios, specificity, carriers and channels, coupling phenomena, isotope interaction, and primary and secondary active transport. Some examples indicating the range of specific coupled systems discussed are the ATPases (Ca^{2+} -activated, Na^{+} - K^{+} -activated, and proton translocating), the redox proton pump, and the phosphotransferase systems. For many readers the brevity of the treatment will be a great advantage, and the ready accessibility of a large number of formulae to suit every occasion (and taste) may well be, for some, the most attractive feature of the book.

However, this is by no means an eclectic account of transport bioenergetics. Quite the opposite is the case: it bears the unmistakable stamp of a single school. To readers familiar with kinetics this may not

be uncongenial. The author places great emphasis on the so-called "Law of Mass Action" treatment, in which transport phenomena are formally described as if they were chemical reactions. The implication is that coupled processes such as active transport are intrinsically stoichiometric, that is, completely coupled, and that any slippage must be attributed to internal or external leakage, or to "leakage" of the driving reaction in the case of a pump. This view does not comprehend the wider possibility that incomplete coupling may be a fundamental property of the system and even confer on it some regulatory advantages. Nevertheless, a serious attempt has been made to combine and integrate the kinetic and thermodynamic analyses. It might have succeeded better if certain lapses in rigour had been eliminated — for example, the present reader was surprised to find the standard affinity being put forward as the effective driving force in active transport, and to be informed that completeness of coupling implies an efficiency of 100%.

Although the author has certainly provided a unified and consistent exposition

of his theme, lacunae occur which are troublesome. Some of these are to be anticipated from the preface, in which he states: "The simplifying assumptions may often be unreal. Hence the equations should not be applied uncritically . . .," and later ". . . no attempt has been made to quote the underlying literature completely and according to priority". Indeed, the sparing use of references will undoubtedly make critical evaluation of the equations a hard task for the tyro. Perhaps the most important omission is the absence of experimental data which might have been used to illustrate the usefulness and range of applicability of the theory.

The book is marred by the usual sprinkling of misprints, but is handsomely bound, as behoves a manual to be thumbed through at frequent intervals. With some reservations I recommend its purchase, suspecting that its utility will be greatest for confirmed *aficionados*.

S. R. Caplan

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Semiconductor systems

Problems of Linear Electron (Polaron) Transport Theory in Semiconductors. By M.I. Klinger. Pp. 931. (Pergamon: Oxford, 1979.) £62.50.

THIS is a very large and expensive addition to Pergamon's Natural Philosophy series, being some nine hundred pages in length. It covers the theory of linear transport and other properties of electrons in a range of semiconductor systems which become gradually more disordered as the book proceeds (reflecting the research trends of the past three decades). As one might expect in such a large book on a restricted topic, there is a vast amount of detail, with every major (and in some areas minor) theory described.

The first two chapters form an introduction to transport and solid-state theory. The interaction of an electron with the lattice vibrations to form polarons is then described in various mathematical and physical approximations. The dynamic and transport properties of the polaron in crystals are considered in the following three, very long, chapters. The final part of the book attempts to draw together the theories of electron conduction in disordered semiconductors.

The content of the book is, in one respect, excellent, in that almost everything one could wish for has been covered concerning the theoretical development of the subject up to the mid-1970s. It is not a textbook in the nor-

mal sense; however, there is a strong tendency to present the problems and the various solutions with little explanation. In some areas it was necessary to go back to the original papers to understand the author's over-concise version. Throughout, the emphasis is on the formalistic development of the subject where possible, experiment playing only a very minor role. Even with this omission, the treatment is very condensed and mathematical, especially in the first half of the book. In consequence, however, a large range of properties and physical models have been covered.

Very few concessions are made to the reader: a very high mathematical ability and a good background knowledge are assumed, making this a book for the theoretical specialist rather than the general research worker. In a field where experiment and simple physical models have played such a large part in aiding our understanding, especially in the disordered systems, this is unfortunate and gives a distorted view.

In conclusion this is a rather lopsided book; a more critical approach to the content could have reduced the size and still have allowed room for a more balanced treatment of the subject. What one has here is a very specialised reference book, undoubtedly very useful to a restricted number of people but not really fulfilling the need of the many experimental workers in this field.

J.C. Inkson

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Materials science

Science of Materials. By W. Brostow. Pp. 436. (Wiley-Interscience: Chichester, UK, and New York, 1979.) £20.

THIS book is intended as an undergraduate text in materials science, but differs significantly from existing books in its approach. This difference can best be summarized by saying that Professor Brostow has given us a physicist's view of materials science rather than a materials scientist's! Thus the solid state is not introduced until the seventh chapter, the first six being devoted to mathematical techniques, statistical mechanics, intermolecular forces, gases, liquids and solutions.

This early portion of the book is excellent, especially the treatment of statistical mechanics which is rarely introduced in any depth in materials texts. The treatment of intermolecular forces is also refreshing, escaping as it does from the simplistic classification of chemical bonds usually presented in books for undergraduates.

The amount of space devoted to the physics of matter means that some essential topics such as phase equilibria, crystal structure and defects are treated in a very basic fashion, appropriate only to a first-year level. This tendency to touch on subjects without dealing with them adequately increases as the book proceeds. Thus, polymeric materials are covered completely in eight pages (although rubberlike elasticity provides a further six pages in a later chapter.) Composite materials have a separate chapter, but more than half of its twelve pages are devoted to wood, while fibre reinforcement is dismissed in less than a single page. The mechanical properties of all forms of solids and liquids occupy a single chapter and strengthening mechanisms in crystalline solids receive only a three-page coverage. Electrical and dielectric properties are treated at what seems to be a sub-degree level, and a final chapter on the testing of materials contains only four pages of text and no diagrams.

In a book of manageable size, of course, it is quite impossible to achieve both depth and breadth. Professor Brostow has settled for depth in the physics of matter and breadth in materials science, and this balance will no doubt appeal to teachers of physics. By the same token, however, it will not satisfy the needs of materials and engineering undergraduates. The high cost of the present hardcover edition will also discourage its use as an undergraduate text.

E.H. Andrews

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obituary

Dennis Gabor, 1900-1979

DENNIS GABOR, who received the Nobel Prize for Physics in 1952, for the invention of holography, died on 9 February 1979. One can surmise that he would have approved of such a one-sentence summary — holography was the crowning achievement, contemplated with great pleasure. Yet, it gives a distorting emphasis on a man who contributed radically new ideas to a number of quite distinct areas of applied physics, and who moved with effortless facility across the cultural divide.

Gabor was born in 1900 in Budapest, where he spent all his school days and completed three years at the Technical University. At the age of 21, he moved to the Technische Hochschule in Berlin, where, after receiving his Diploma, he commenced his doctorate studies under Professor Mathias. These centered on the use of cathode ray tubes for the observations of transient waveforms — thereby initiating a development, which has continued to this day, always in pursuit of the faster transients that have marked the path of progress in electronics. A by-product of this research was the development of the first electron lens; it had been known for some time that electrons could be focused by means of a 'concentrating coil', but it was Gabor's use of a magnetic circuit to concentrate the magnetic field over a short region which led to the first practical lens. Although he did not, at the time, fully understand its mode of action (it was just before the celebrated paper by Busch which marks the beginnings of modern electron optics), the invention was to play an important role in the realisation of the first electron microscope. In fact, the first demonstration of such a microscope by Borries and Ruska used the actual lens created by Gabor.

Gabor had himself speculated on the possibility of realising an electron microscope, as he has recounted in a fascinating article on the History of the Invention of the Electronmicroscope (*Elektrotech. Z. Ausg. A*, 78, no. 15, 522-30, 1957) — but enthusiasm for undertaking the development was dampened by the expectation that any objects of interest would be rapidly carbonized by the electron beam. By the time Borries and Ruska embarked on the venture, Gabor had moved to the Siemens



Research Laboratory.

It was a narrow escape from greatness at an early age. Perhaps that early disappointment served to sharpen his subsequent enthusiasm for the inventive process; Shockley has attributed his invention of the junction transistor to his chagrin at having missed the earlier point-contact device.

The work at Siemens was largely devoted to a new type of gas discharge lamp. Whilst this research failed to lead to a commercially exploitable product, it marked the beginning of a life-long interest in the plasma state and its applications. It also led to a move to the Central Research Laboratories of B.T.H., Rugby, in 1933 — Gabor's response to the election of Adolf Hitler in Germany. The move to Rugby was facilitated by inspired talent-spotting on the part of Professor T. E. Allibone.

While continuing his work on gas discharge lamps, Gabor never entirely abandoned his interest in electron optics. He invented what we would now call a mechanical analogue computer for electron trajectory tracing; coincidentally with Irving Langmuir, he discovered the basic limitations on electron density in electron optical systems.

During the war, by reason of his status as an alien, Gabor was not able to contribute

to the main stream of research — it was a time when the damning fact of foreign origin was more than a match for rational assessment. He was, nonetheless, able to make pioneering contributions to infra-red imaging and to develop a deeper understanding of the interaction between electrons and electromagnetic fields, the latter leading to a series of classic papers published in the last two years of the war. It also enabled him to continue work on electron optics and, above all, to do battle with what he perceived as the key problem — how to improve the quality of electron optical lenses. The constraints imposed by Laplace's equation deprive the electron optical designer of most of the weapons which are available in classical optics. The aberrations of electron optical lenses seemed to bar the path to the achievement of the ultra high resolution of which electron microscopes should have been capable — a resolution which could image single atoms.

Gabor tried to find room for manoeuvre by suggesting the introduction of space charge into lenses, to correct the aberrations — a technique which, as it eventually turned out, could not be effective, on account of the blurring occasioned by direct electron-electron interaction. Then, in 1947, quite suddenly, he perceived that the way to deal with bad lenses was not to strive to make them a little better, but, rather, to get rid of them altogether. The strategy envisaged by Gabor was made up of a number of quite distinct and quite brilliant insights — the ability to record the phase of a wavefront using materials sensitive only to intensity, by including a 'reference' wave; the possibility of recovering the information contained in the original wave by a reconstruction process with another, complementary wave; the recognition that the recording and reconstruction waves need not have the same wavelength — that an electron 'hologram' might be reconstructed at optical wavelengths. Gabor was able to demonstrate the principle at optical wavelengths, using the most coherent source then available — a mercury arc illuminating a pin hole. He published the results in a celebrated communication to *Nature* in 1948. It was a long way from the bold scheme envisaged for improving electron micrographs;

indeed, to this day, such improvements have not been realised — though the hope of eventual success is not dead.

Gabor then developed a comprehensive theory of holography, but few more experiments were conducted for a dozen years. It was not until the invention of the laser provided experimenters with highly coherent sources that holography had its second birth, in a series of inspired researches undertaken by Leith and Upatnieks.

The subsequent story of holography is one of explosive growth. It is now the basis of a large and growing industry; it has inspired new forms of art. It is still far too early to assess its ultimate impact on science, technology and the conduct of human affairs.

How should such a discovery be weighed and judged? Gabor, himself, wondered how one should assess the value of a discovery in applied science, in the aforementioned paper on the history of the electron microscope. The following is a translation from the German of one of the opening paragraphs in that paper:

'It is hardly to be debated that those whose work is most highly regarded are those who have pioneered a new field which has subsequently been shown to be fertile. In this there is an element of luck, possibly even of injustice; honours are not bestowed — as they are, for example in mathematics — in proportion to the mental effort required to reach the result. In fact, I would go further and suggest that if a scientific achievement is to receive the highest accolade, it should not be wholly rational; there must be some element of boldness and risk. We regard the discovery of electromagnetic waves by Hertz as a greater achievement than Hamilton's theory of conical refraction not only because Hamilton's work put the finishing touch to the theory of crystal optics whilst in contrast, Hertz opened up a wide and new field, but also because, when Hertz began his classic investigations, Maxwell's theory was by no means established; moreover, there was not the slightest ground for the expectation that the conductivity of the electric spark could decay in 10^{-9} seconds. This was a matter of boldness and risk — not one of logical expectation.'

The passage was written with reference to electron microscopy. It was 1957, and Gabor certainly had not the slightest expectation that holography could ever be viewed in such terms. Certainly, the research students he acquired when he moved to Imperial College as a Reader in 1948 — and these remarks are written from the vantage point of one of these — regarded holography as a laboratory curiosity. Yet, with hindsight, the passage is, surely, an entirely apt commentary on the invention of holography.

Often one finds that an inventive step follows very hard on the heels of another discovery, or some advance in technology

which made it possible. Holography, however, was ready for birth at least half a century before the idea occurred to Gabor. Moreover, although the demonstrations in optics were difficult, there was no inherent reason why the principle should not have been demonstrated in acoustics — where, indeed, it has found application in recent years. By this criterion also, the invention of holography must be seen as an extraordinary stroke of brilliance.

In this period, Gabor also produced his pioneering contributions to what we now call communications theory — beginning with a remarkable paper published in the *Journal of the Institution of Electrical Engineers* in 1946. This work was inspired by the desire to gain a deeper understanding of the structure of information transmission; yet, included in the motivation there was, as always with his work, a thrust towards the applied, towards the inventive step. It led him to investigate means for transmission of information using 'band-width compression', a theme which remains an exciting area of research to this day.

When Gabor moved to Imperial College in 1948, he presented a remarkable and, at first, unfathomable phenomenon to his research students. He had little comprehension of the difficulty encountered by ordinary mortals in attempting to gain an appreciation of his aims in a particular research endeavour, or to follow a leaping thought line without the help of stepping stones. His lecture courses on electromagnetic theory, statistical physics, the plasma state, electron optics, seemed memorable but hardly capable of assimilation — at least, until the realisation dawned that they were not so much lectures as master classes. It was necessary to 'know' the subject before attending these occasions; but then the experience was enormously worthwhile — providing both the long historical view as insight in depth and generality. There must be many who still treasure the copious lecture notes typed at high speed by Gabor himself using an amazing two finger technique on a battered, ancient portable. There must be many who regret that these notes never found a more permanent expression in what would have amounted to a unique set of books; but research, invention and original thought was always the greater attraction and the more compelling master of his time.

There was no doubt amongst his students that Gabor could and should receive the Nobel Prize for something — we debated just what it might be. Nor was this a symptom of hero worship, of which we were largely innocent. We could assess the magnitude of the intellect but found it harder to discern its direction. We were also aware of a personality of great warmth yet of inherent shyness. Communication was not always easy. And the attempts to capture that personality, the highly charged, penetrating comments, the

humour, the perfection and elegance of expression, though clothed in a Hungarian accent of some substantial depth, was the supreme task of those endowed with any ability of mimicry — though one doubts whether their skill will ever again be displayed.

Recognition outside his immediate group was slow to come. Gabor was a physicist who saw himself as a practical engineer — a reversal of the more usual aspiration for role changing. Not all physicists recognised this situation. I remember a seminar, perhaps around 1953, to an audience largely of physicists, where Gabor discussed the interpretation of discrete energy losses suffered by electrons when transmitted through thin metal films, in terms of plasmon excitation — an explanation which we now know to be essentially correct. The criticisms which he met at the time were fierce, and seemed to transcend the requirements of scientific debate — there seemed to be an element of a demarcation dispute.

By the early fifties, Gabor had completed his work on holography, made pioneering contributions to information theory, to electron optics, to electron-electromagnetic interactions and to plasma theory. Yet, he was still a Reader in the University and repeatedly failed to achieve election to the Royal Society. The omission was resented by his students and others who knew him well; it was deeply felt by Gabor himself. It was hard to comprehend at the time; it is no easier to do so in retrospect. Throughout this period, however, Gabor's creative work never faltered. Flat television tube research involving the most ingenious electron optical conceptions lived side by side with learning machines, with holographic microscopy and, a little later, with schemes for direct generation of electricity from heat using a plasma — a technique which echoed some of his work on gas discharge lamps of three decades earlier. Many of these schemes remain as fascinating concepts, devoid of practical implementation. But it would be unwise to write them off; holography had a gestation period of 15 years.

In 1956, Gabor was, at long last, elected a Fellow of the Royal Society and two years later became the Professor of Applied Electron Physics at Imperial College. His inaugural address marked a totally new direction in his publications and his thought, — to the socio-political problems faced by mankind as the direct consequence of advances in technology. The lecture, later published in the form of a most compelling contribution to *Encounter*, eventually formed the basis of a celebrated book, *Inventing the Future*. The trilemma facing mankind: war, excess population and leisure, is now revealed wisdom — part of the backcloth of our existence. It was not so when the book was first published in 1963. The approach taken in this book — an appraisal of the

enormous dangers, coupled with a guarded optimism that, with their recognition, the worst could be avoided — Gabor pursued and developed in a series of further publications. He became a founder member of the Club of Rome, and thereby became embroiled in the fierce debates which followed the publication of *Limits to Growth* by Forrester and Meadows. His stance was one of cheerful and total support for these authors — though a careful examination of his expressed opinions reveals that this support did not, in fact, rest on his assessment of the computer model calculations which caused such widespread alarm.

Just before *Inventing the Future*, it became clear that holography, transformed by the discovery of the laser, was turning into one of the most rapid

growth topics in applied science. The range of applications appeared to grow month by month; holography was recognised as a principle of such generality that it could touch many branches of physics, biology and engineering. Gabor, himself, contributed several inventions to this growing field. He lectured widely on the subject. Many will never forget a brilliant discourse at the Royal Institution which he gave in 1969. The recognition, so long denied, or belatedly bestowed, came in full flood. Honours of all kinds were showered upon him and, in 1972, the Nobel Prize.

There remained some good years. They were devoted to scientific work at the CBS Laboratories in the USA, to Imperial College and London, and to a villa in Italy. Gabor continued to contribute to what became his dominant and passionate

concern — the adjustment of the human condition to the new world, which the explosive development in science and technology had wrought within his own lifetime and experience. Prophecy is the hardest of arts; scientists as a body are not notably more successful than others in its practice. Yet, if any doubt that there are some, a very few, who can penetrate that ultimate unknown at least some little distance, they should re-read *Inventing the Future* of 1963, or, perhaps, the Fawley Lecture of 1972, *The proper priorities of Science and Technology*.

Dennis Gabor married Marjorie, née Butler, in 1936, a marriage which brought great happiness and continuing support, in a life which knew both bitter disappointment as well as triumph and total fulfilment. *Eric A. Ash*

Airey Neave

A GENERAL ELECTION is a deep parliamentary gulf dividing sharply one time from another. I have returned to the House of Commons not only to a new parliament and a new government but to unfamiliar faces that crowd the benches as solidly and confidently as did those whose places they have taken. But there is outstandingly one face absent from the chamber, one expected figure not again to be met in the long corridors. I refer to my friend Airey Neave, the victim of a cowardly political murder within the precincts of that same Palace of Westminster where we meet.

Leading figures in public life, the newspapers, radio and television have all said so much deservedly in tribute to Mr Neave, his life and work, that it might seem presumption on my part to add more. Nevertheless these columns give me an opportunity to enlarge a little on his considerable services to the Select Committee on Science and Technology of the House of Commons, for Airey Neave was a founder member of the committee from its establishment in 1967 until 1975 when he became opposition spokesman on Northern Ireland.

I was the first chairman of the select committee but in 1970 when the Conservative government of Mr Heath came to power, in accord with parliamentary practice, Airey Neave succeeded me for the parliamentary sessions of 1970-1974. Every chairman, I suppose, has his own style and Airey's was very much his own. In dealing with witnesses, especially those (and there are a few) who think that politicians cannot possibly have knowledge of anything except the suspect business of getting elected, he could be severe to near inquisitorial. Yet never for an instant did he go beyond the needs of the inquiry being conducted. As a good select committee chairman should, he saw his primary duty to parliament, not to governments or parties. I remember well how he gave a rising Conservative ministerial star caught out in a mistake a wiggling that led to with-

drawal and public apology. But to the general run of helpful and co-operative witnesses, enduring the unfamiliar ordeal of questioning in public, Airey was the soul of courtesy.

In the chair Airey Neave was vastly impressive to witnesses as he was to colleagues on the committee because of his detailed knowledge of the subject under consideration. His capacity for work was truly prodigious. Among the inquiries he handled either as chairman of the main committee or as chairman of a sub-committee, were population control, the Rothschild proposals and the computer industry.

But I suppose the inquiry that brings me closest to Airey Neave in memory was the one that the select committee carried out, through a sub-committee, into the choice to be made by Britain for the 'third generation' of nuclear reactors, the Magnox reactor being counted first generation and the advanced gas-cooled reactor second generation. This was during the time of the Heath government, when Mr Peter Walker was, as Secretary of State for Technology, the responsible minister.

The assumption had been that no firm decision would be taken on the choice to be made without a ministerial statement and the normal opportunity for parliamentary questioning. Then a long special article appeared in a leading Sunday newspaper by a science correspondent, to the effect that the Central Electricity Generating Board had made up its mind already and that American style pressurised water reactors were to be bought on a large scale for installation in the British electricity supply system, with the co-operation of our manufacturers and the apparent approval of the government.

Simple parliamentary questioning took us little further forward and Airey and I both agreed that this was just the kind of situation for which the select committee had been established. After all, the British taxpayer had either spent or had guaranteed large sums of money in the past on the assumption that Britain had the most efficient and safest nuclear power arrange-

ments in the world with the gas-cooled systems and now for parliament to be told that we had been wrong all the time took more than a little swallowing.

Because of his declared interest as a director of a plant manufacturer engaged in the nuclear field, Airey Neave felt he should not take the chairmanship of the inquiry and I occupied his place, though he served on the sub-committee. To a great extent it was the safety of the PWR in an emergency situation that took the attention of the inquiry and on this point critical evidence was received from Sir Alan Cottrell, then Chief Scientist to the government — evidence which had not been published before.

The conclusion of the select committee, reported to the House of Commons, was that the case for abandoning British reactor systems and adopting the American system could not be sustained by the evidence submitted and we advised caution over the PWR. Needless to say, some but not all parts of the nuclear technological establishment attacked the select committee for political meddling and worse. Nevertheless, a full day's debate in the Commons found members of parliament on both sides impressed by the committee's findings and Airey and I followed up the debate with a joint appearance on the national television network which engendered a large correspondence. Many of our correspondents were obviously impressed by the way in which a select committee of the Commons could, while supporting nuclear development in general, run up a danger flag about one particular proposed development. Who in the light of Harrisburg could to-day say we were wrong?

I spoke to Airey Neave last on the Thursday night before his death on the following day. We met by chance in the Commons lobby and discussed the future of the select committee on Science and Technology in the light of suggested procedural reforms for select committees in a new parliament. I did not know then I was never to see him again.

Arthur Palmer

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